COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: Effect of Dapagliflozin on Hepatic and Renal Glucose Metabolism Subjects

NCT number: NCT02981966

IRB Approval Date: 10/18/19

Unique Protocol ID: HSC20160596H

Two groups of subjects (age = 18-70 years; BMI = 21-45 kg/m^2; males or females) will participate: (i) 24 healthy NGT individuals, and (ii) 24 T2DM subjects according to the ADA criteria. NGT and diabetic subjects will be matched for age, sex and BMI. Diabetic subjects must be on a stable dose (more than 3 months) of monotherapy or combination therapy with metformin and/or a sulfonylurea and have HbA1c <8.0%. Subjects taking drugs known to affect glucose metabolism (other than metformin and sulfonylurea) will be excluded. Other than diabetes, subjects must be in good general health as determined by physical exam, medical history, blood chemistries, CBC, TSH, T4, EKG and urinanalysis. Only subjects whose body weight has been stable (± 3 lbs) over the preceding three months and who do not participate in an excessively heavy exercise program will be included. Individuals with evidence of proliferative diabetic retinopathy, plasma creatinine >1.4 females or >1.5 males, or 24-hour urine albumin excretion > 300 mg will be excluded. Diabetic subjects will be recruited from the Texas Diabetes Institute (TDI) in San Antonio (Deputy Director = Ralph A. DeFronzo, MD). TDI is the largest institute in the United States that provides comprehensive care for ~ 10,000 unduplicated T2DM patients. Therefore, we do not anticipate any problem in recruiting the required number of patients for this study. NGT subjects will be recruited from volunteers who have participated in prior studies.

Following a screening visit to determine eligibility, NGT subjects will receive a 75-gram OGTT to confirm glucose tolerance status. In all subjects, the rate of whole body (liver plus kidney) and renal glucose (renal vein catheterization with 3-^{3}H-glucose) production will be measured before and after dapagliflozin or placebo. T2DM and NGT subjects will be randomized 2:1 to receive dapagliflozin (n=16) or placebo (n=8).

The reason for including both NGT and T2DM subjects is provided below. In NGT subjects who ingest an SGLT2 inhibitor, urinary glucose excretion promptly increases and amounts to ~72 grams/day (58,59), yet the fasting plasma glucose concentration does not change. This means that endogenous glucose production (kidney plus liver) must increase to precisely match the rate of renal glucose excretion. Therefore, from the quantitative standpoint this will provide the greatest stimulus to augment EGP and, thus, the greatest ability to define the role of the kidney in the increase in EGP. Because, in T2DM subjects, EGP turns on long before the fasting plasma glucose concentrations return to normal, the increase in EGP (and, therefore, any increase in renal glucose production) will be less than in the NGT individuals. Nonetheless, we are very interested in the contribution of the kidney to the increase in EGP in T2DM subjects following dapagliflozin. Therefore, we have included both NGT and T2DM subjects.

Visit 1: Screening. Medical history will be obtained. EKG and physical exam will be performed. Blood will be drawn for FPG, routine blood chemistries, CBC, PT, lipid profile cogulation tests, HbA1c, and thyroid function. Urinanalysis, albumin/creatinine ratio and pregnancy test will be performed. NGT subjects will receive 75-gram OGTT to confirm their glucose tolerance status; plasma glucose, insulin, glucagon and C-peptide conc will be measured before (-30, -15, 0 min) and every 15 min for two hours after glucose ingestion. T2DM subjects will not receive OGTT. An interim visit might be needed for pre-cath evaluation and drawing of coagulation tests.

Visit 2: The rate of whole body (liver plus kidney) and renal glucose production will be measured with 3-^{3}H-glucose in combination with renal vein catheterization. On admission date, subjects will report to the CRC at 6 AM after a 10 hour overnight fast. At 7 AM a catheter will be placed into an anticubital vein and a prime (40 uCi x FPG/100)-continuous (0.4 uCi) infusion of [3-^{3}H]-glucose will be started and continued until 2 PM (4 hours after dapagliflozin or placebo administration). At 8 AM subjects will transfer to cath lab from BRU for renal vein catheter insertion via the femoral vein, under fluoroscopy, or jugular vein, guided by ultrasound. The determination for using the jugular or femoral vein will be under the expertise of Dr. Robert Chilton (examples: body build, accessibilty to femoral vein, circulatory system anatomy, safety concerns with using the femoral vein). Catheter placement will be confirmed by injection of a small amount of contrast material. The renal catheter will be continuously infused with a small amount of saline to maintain its patency. Addtionaly, a second catheter will be placed in the radial artery by Dr. Chilton for withdraw of arterial blood. After finishing these procedures the subjects will be transferred back to the BRU. At 7 AM a prime (8 mg/kg)-continuous (12 mg/min) infusion of para-aminohippuric acid (PAH) will be started for determination of renal blood flow. After 3 hours of tracer equilibration time in T2DM (2 hours in NGT subjects) blood samples will be drawn from the renal vein and arterial catheters at -30,-20, -10,-5 and 0 (time zero is drug [dapagliflozin or placebo] ingestion time) minutes. At 10 AM (time zero), subjects will receive dapagliflozin (10 mg) or placebo and blood samples will be obtained from the radial arterial and renal vein catheters every 20 minutes from 10 AM to 2 PM. Plasma glucose, insulin, C-peptide, glucagon, cortisol, growth hormone, catecholamine, and PAH conc and [3-^{3}H]-glucose specific activity will be measured in blood samples drawn from arterial catheter. The plasma glucose conc and [3-^{3}H]-glucose sp act will be measured from the renal sample. Addtionally, 3 arterial and 3 venous samples at baseline and at the end of study will be drawn to measure PO2 and O2 sat, with an iSTAT handheld analyzer. We will also draw blood

to monitor kidney function. Urine will be collected from 7 to 10 AM and from 10 AM to 2 PM. Urinary volume and urinary glucose and PAH conc will be measured to obtain urinary glucose excretion and to measure renal plasma flow (PAH clearance). The study will end at 2 PM and there will be a 3hr observation period before subjects are discharged at 5 PM. Subjects will be asked to collect a 24- Hour Urine Collection to determine creatinine excretion after either Visit 1 or Visit 2.

Renal oxygenation plays a key role in the pathophysiology of chronic kidney diseases progression. To date, the validated methods for quantitively measuring kidney oxygenation in humans use invasive catheters/biopsy. Direct arterial-venous kidney oxygenation measurement is available only at a small number of research sites. The availability of a clinically practical oxygenation measurement will find immediate applications in many kidney hypoxic diseases including diabetic nephropathy, kidney artery stenosis, and acute kidney injury.

Visit 2B: Subjects will be asked to come back to BRU for follow up and recovery status within 7 days after Visit 2.

Visit 3: Renal (Kidney) MRI: A renal MRI will be performed on subjects for the measurement of kidney size, and whole-organ metabolic rate of oxygen (MRO2). Before entering the MRI scanner, the subject will be surveyed for magnetic articles on his/her person or clothing and briefed regarding the safety requirements of the MRI study. This MRI procedure is entirely non-invasive.

Data Analysis and Statistical Methods: ***The primary end point*** is the difference in EGP (total, renal and hepatic) (see below) during the last hour of study (180-240 min) between subjects receiving dapagliflozin versus placebo. The whole body rate of EGP will be calculated from the 3-^{3}H-glucose infusion as previously described (10). Under steady-state postabsorptive conditions, the basal rate of whole body endogenous glucose appearance (Ra = bEGP) equals 3-^{3}H-glucose infusion rate divided by steady state plasma tritiated glucose specific activity. After drug administration, non-steady conditions for 3-^{3}H-glucose specific activity prevail and the rate whole body of glucose appearance (Ra) is calculated from Steele's equation (57). Ra equals the rate of EGP after drug administration. Ra will be calculated for each time point after dapagliflozin administration and will be plotted versus time and compared to Ra in placebo-treated group.

Renal Glucose Production (RGP) will be calculated as previously described (27). Renal plasma flow (RPF) is calculated as the clearance of PAH; renal blood flow (RBF) is calculated as: RBF = RPF/(1-hematocrit). Net Renal Glucose Balance = RBF X (Art_{glu}-RV_{glu}). Renal glucose fractional extraction (FE) is calculated from tritiated glucose radioactivity as: FE= (A_{dpm}-RV_{dpm})/Art_{dpm} and Renal Glucose Uptake = RBF X FE X $[Glucose]_{art}$. From the following equation: Net Renal Glucose Balance = Renal Glucose Uptake – Renal Glucose Production, one can calculate Renal Glucose Production (RGP) as: RGP = (RBF x FE x $[Glu]_{Art}$ – (RBF x $[Glu_{Art\text{-}RV}]$). HGP is calculated as Ra(total) - RGP. The effect of dapagliflozin versus placebo on RGP and HGP will be calculated as the difference in each parameter (RGP and HGP) at each time point minus the mean value before drug administration.

Values will be presented as mean $\pm$ SD. The change in EGP and RGP after dapagliflozin and placebo will be compared in the two groups (NGT + T2DM) with repeated measures ANOVA with time and treatment group as factors. Only subjects who complete the entire protocol will be analyzed.

Anticipated Results and Potential Limitations:

The results of the present study will identify the organ, liver and/or kidney, responsible for the increase in EGP following dapagliflozin administration. Our published results (10) demonstrate that the decrease in FPG concentration (43 mg/dl) following two weeks of dapagliflozin treatment in T2DM subjects was associated with an increase in EGP (0.45 mg/kg.min). Because inhibition of SGLT2 in healthy NGT individuals is not accompanied with a significant change in the FPG concentration (58,59), we anticipate that the increase in EGP in NGT individuals will be even greater than in T2DM individuals. Based on the amount of glucosuria produced with SGLT2 inhibitors in NGT individuals (72 grams/day) (58,59), in order to compensate for the total renal glucose loss, we anticipate that the rate of EGP will increase following dapagliflozin by ~0.7 mg/kg.min above baseline. The contribution of the kidney to whole body glucose production is ~20-25% (26). Since whole body EGP under basal conditions is ~2 mg/kg.min (2), the contribution of renal glucose release to EGP is ~0.4 mg/kg.min. Thus, the kidney has to double, or even triple, its rate of glucose production in NGT individuals in order to account for the increase in total EGP triggered by glucosuria. Moreover, published results indicate that renal glucose production is not responsive to an increase in plasma glucagon concentration (29,30). In contrast, insulin and glucagon are powerful regulators of HGP (6,17,18). Therefore, we anticipated that, at least in part,

an increase in HGP secondary to the rise in plasma glucagon concentration and decrease in plasma insulin concentration in response to dapagliflozin-induced glucosuria (Figure 1) will account for the majority of increase in EGP in both NGT and T2DM subjects (see discussion below). However, it is possible that local effects of SGLT2 inhibition stimulate renal glucose production (60,61) without the need for a hormonal signal. Measurement of renal glucose production will provide a direct and definitive answer to the contribution of the kidney to the observed increase in total EGP following SLGT2 inhibition.

A potential study limitation is the high rate of renal blood flow, which could make it difficult to detect a small increase in renal glucose production. However, if all of the increase in EGP (0.5-0.7 mg/kg.min) following dapagliflozin originates from the kidney, this amount of glucose production should cause at least a 20% decrease in renal vein specific activity, which easily is detectable by the current methodology. Even if half of the increase in EGP (0.35 mg/kg.min) following dapagliflozin originates from the kidney, the current methodology is sensitive enough to detect a 100% decrease in renal vein specific activity. However, an undetectable change in enhanced renal glucose production following SGLT2 inhibition would not completely rule out a small contribution of renal glucose production to the increase in EGP in response to glucosuria.

Sample Size Calculation: In our published results (10), the mean basal rate of EGP in T2DM subjects was 2.20±0.24 mg/kg.min and the mean difference in EGP during the last hour following dapagliflozin versus placebo was 0.70±0.34 mg/kg.min. We computed that 24 T2DM subjects (2:1 randomization; 16 dapagliflozin and 8 placebo) and 24 NGT subjects (2:1 randomization; 16 dapagliflozin and 8 placebo) are required to result in a combined analysis (T2DM + NGT; 32 dapagliflozin and 16 placebo) with 90% power to detect a 0.35±0.34 mg/kg.min (which equals 50% of the increase in EGP caused by dapagliflozin) increase in renal glucose production at alpha <0.05. (PASS Version 11, NCSS, Kaysville UT 2011). All statistical calculations were performed by Joel Michalek, Professor, Dept. of Epidemiology and Biostatistics, UTHSCSA).

Risk Benefit

(4) Potential risks include the following:

a) Blood withdrawal. All studies involve the withdrawal of blood. Total blood drawn during the study will not exceed 500 ml, or one pint of blood over a 4 week period. We do not believe that this amount of blood loss will pose any risk to the subject's health. Subjects will be questioned about their history of blood donation and subjects who have donated blood in the previous two months will not be studied. The subjects will be told that they should not donate blood for two months after the study. Blood hematocrit will be measured in every subject during the screening visit and any subject with a hematocrit of less than 34% will not be studied.

b) Renal Vein Catheterization. During renal vein catheterization a catheter will be inserted into the femoral vein, under fluoroscopy, or jugular vein, with guide by ultrasound, and , passed into the renal vein. Local complications of this procedure is pain/discomfort in the insertion area (~10-15%), black/blue discoloration (~5%), hematoma (<1%), and renal vein thrombosis (<0.1%). Should the latter occur, the clot would have to be removed with a declotting catheter using fluoroscopy. To reduce the risk of this complication, the renal vein catheter will be kept patent with a slow drip of normal saline. During the initial catheter placement in the renal vein, there will be a small amount of radiation exposure. Because the time required for placement of the renal vein catheter is short, 2-4 minutes, the radiation exposure is small. A risk of using the jugular vein for catheter insertion is a pneumothorax or puncture of the lung. The risk of a pneumothorax occurring is less that 1 out of 100 as we will be using ultrasound for insertion of the catheter.

c) Radial Arterial Catheterization. During the renal vein catheterization study, a catheter also will be placed in the brachial artery to obtain an arterial blood sample. Complications of this procedure include local pain (~10-15%), black-blue discoloration (~5%) and hematoma (<1%). To prevent hematoma formation, local pressure will be applied for 5 minutes after removal of the catheter and a pressure bandage will be applied for 24 hours. Clot formation in the brachial artery is uncommon and occurs in <0.1%. if this should occur, the clot would have to be removed with a declotting catheter or surgically. To prevent hematoma formation, the catheter will be kept patent with a low saline infusion.

d) IV lines. Catheters will be placed in an anticubital vein and a hand vein for the measurement of glucose production. Local hematomas occur in about 1% of catheterization. Infection is possible

(<1%), but we have not experienced this complications. One instance of thrombophlebitis has been observed (<0.1%). The hand with the catheter will be placed in a warm (65°C) transparent plastic box to arterialize venous blood. We have observed one instance of skin burning (2nd degree) using the heated box (<0.01%). Subjects will be informed to tell us if their hand feels excessively warm or uncomfortable.

e) Tritiated glucose is given during these studies. The radiation exposure from tritiated glucose is well within guidelines (Shreve WW et al. Proc 2nd International Conference on Peaceful Uses of Atomic Energy, Geneva, 1958; U.S. Department of Commerce National Bureau of Standards Handbook 69, 1969). The cumulative radiation exposure also is well within the dose range (1,000 µCi) approved by the University of Texas Health Science Center Radiation Committee. The Radiation Safety Committee will approve these studies before anyone is enrolled. At these low exposures, risk is minimal.

f) The procedures for minimizing risk are listed above in context with the specific risks.

g) Information learned about all subjects will be kept confidential and handled in strict accordance with HIPAA guidelines. Subjects will not be identified in any way in any publication. With the precautions outlined above, the risk/benefit ratio in the study is very low.

h) Serious hypersensitivity reactions to dapagliflozin have been described. Anyone with a history of hypersensitivity reaction to dapagliflozin will be excluded.

i) Diabetic subjects with severe renal impairment (eGFR < 45 ml/min), end-stage renal disease, or dialysis will be excluded.

j) Subjects with uncontrolled hypertension (BP > 160/100 mmHg) will be excluded; subjects with orthostatic hypotension (decrease in BP > 15/10 mmHg) when standing will be excluded.

k) Ketoacidosis

The U.S. Food and Drug Administration (FDA) has warned that dapagliflozin may lead to ketoacidosis, a serious condition where the body produces high levels of blood acids called ketones that may require hospitalization.

Patients will be asked to pay close attention for any signs of ketoacidosis and call us immediately if they experience symptoms such as difficulty breathing, nausea, vomiting, abdominal pain, confusion, and unusual fatigue or sleepiness. We will evaluate for the presence of ketoacidosis in patients experiencing these signs or symptoms and discontinue dapagliflozin if acidosis is confirmed and take appropriate measures to correct the acidosis and monitor sugar levels.

l) Urosepsis and Pyelonephritis

Rarely, dapaglilfozin treatment has been associated with bladder and kidney infection. If you experience pain or burning urination, pass cloudy or blood-tinged urine, or experience fever and chills, please call us immediately so that we can examine your urine and obtain a urine culture. We also identified 19 cases of life-threatening blood infections (urosepsis) and kidney infections (pyelonephritis) that started as urinary tract infections with the SGLT2 inhibitors reported to FAERS from March 2013 through October 2014. All 19 patients were hospitalized, and a few required admission to an intensive care unit or dialysis in order to treat kidney failure.

m) Hypoglycemia may occur with dapagliflozin, especially when dapagliflozin is given in combination with a sulfonylurea or insulin. During the study we will measure your blood glucose level frequently to prevent this complication.

n) Genital mycotic (fungal) infections may occur in individuals who take dapagliflozin. They occur

in about 6-8% of females and 1-2% of males. If you have any genital discomfort (itching, burning, discharge), please let us know so that we can give you something to treat the infection.

o) Dapagliflozin can cause an increase in the LDL cholesterol. We will check your LDL cholesterol and if it is greater than 120 mg/dl you will not be able to take part in the present study.

p) If you have a history of bladder cancer or currently have bladder cancer, please let us know, since individuals with bladder cancer are excluded from the study.

Based upon the above considerations, the benefit (knowledge to be gained) to risk ratio is very favorable.

Safety

Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product.
The term AE is used to include both serious and non-serious AEs.

Definitions of serious adverse event

A serious adverse event (SAE) is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

The causality of SAEs (their relationship to all study treatment) will be assessed by the investigator(s) and communicated to AstraZeneca (AZ).

Recording of adverse events

Follow-up of unresolved adverse events
Any AEs that are unresolved at the patient's last AE assessment at the end of the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

The following variables will be collected for each AE:

- AE (verbatim)
- The date and time when the AE started and stopped
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)

- Action taken with regard to investigational product*:* (AE caused subject's withdrawal from study *(*yes or no*)*
- Outcome

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study drug(s)
- Causality assessment in relation to Other medication
- Causality assessment in relation to Additional Study Drugs
- Description of AE.

Causality assessment:

- The Investigator will assess causal relationship between Investigational Product and each Adverse Event, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'
- For SAEs, a causal relationship will also be assessed for other concomitant medications, study procedures, and comparator study drugs. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

Reporting of serious adverse events

Investigators will inform the IRB of the UTHSCSA within 24 hours of any adverse events. Investigators and other site personnel will inform the FDA, via a MedWatch/AdEERs form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations and will concurrently forward all such reports to AZ. A copy of the MedWatch/AdEERs report will be faxed to AZ at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AZ at the same time.

When reporting to AZ, a cover page will accompany the MedWatch/AdEERs form indicating the following:

- Investigator Sponsored Study (ISS)
- The investigator's name and address
- The trial name/title and AZ ISS reference number

Investigative site will also indicate, either in the SAE report or in the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the (PI).
The SAE report and accompanying cover page will be sent by way of fax to AZ's designated fax line: 1-302-886-4114 or via email: AEMailboxClinicalTrialTCS@astrazeneca.com.
Serious adverse events that do not require expedited reporting to the FDA will be reported to AZ using the MedDRA coding language for serious adverse events.
In the case of blinded trials, AZ will request that the Sponsor either provide a copy of the randomization code/code break information or unblind those SAEs which require expedited reporting.
All SAEs will be reported to AZ, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator will be responsible for informing the WCMC-Institutional Review Board (IRB) of the SAE.

Safety assessments

Safety assessments will consist of monitoring and recording all TEAEs, SAEs, AEs leading to discontinuation/withdrawal from study, laboratory evaluation for hematology, blood chemistry, and urine values; pregnancy testing; measurement of vital signs and ECGs; and performance of physical examinations.

AstraZeneca is the manufacturer of the study drug/medication.
Label, storage and distribution are a sponsor's responsibility.

Investigational Products

Description or Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Dapagliflozin 10 mg	Green, plain, diamond shaped, film coated 10 mg tablet	AstraZeneca
Matching placebo for dapagliflozin 10 mg	Green, plain, diamond shaped, film coated tablet	AstraZeneca

The tablets contain lactose, which may cause discomfort in lactose-intolerant individuals.
Dapagliflozin and its matching placebo will be supplied in bottles.

Labeling
The sponsors will label the study medication in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines for labeling (the labels will fulfill GMP Annex 13 requirements for labeling).

Storage
All study drugs should be kept in a secure place under appropriate storage conditions. The investigational product label on the bottle specifies the appropriate storage.

PROTOCOL 1

Time (minutes)	RV Glucose (0.5 ml)	RV PO2/ O2Sat (0.5 ml)	RV 3-3H-glu (4ml)	Arterial Glucose (0.5 ml)	Arterial PO2/ O2Sat (0.5 ml)	Arterial 3-^{3}H-glu (4 ml)	Arterial Insulin/C-Peptide (4 ml)	Arterial Glucagon (2 ml)	Arterial Cortisol (2 ml)	Arterial Growth Hormone (2 ml)	Arterial Cate cholamine (4 ml)	Arterial PAH (4 ml)
-180 (6 AM)			4ml (BG)			4ml (BG)						
		Start 3-^{3}H-glucose and PAH										
-105 (7:15 AM)		Inset renal vein/radial arterial catheter										
-30	X	X	X	X	X	X						
-25		X			X							
-20	X	X	X	X	X	X	X	X	X	X	X	X
-10	X		X	X		X	X	X	X	X	X	X
-5	X		X	X		X						
0 (10 AM)	X		X	X		X	X	X	X	X	X	X
		Administer dapaglilfozin or placebo										
20	X		X	X		X	X	X	X	X	X	
40	X		X	X		X	X	X	X	X	X	X
60 (11 AM)	X		X	X		X	X	X				X
80	X		X	X		X	X	X	X	X	X	
100	X		X	X		X	X	X				X
120 (12 Noon)	X		X	X		X	X	X	X	X	X	X
140	X		X	X		X	X	X				
160	X		X	X		X	X	X	X	X	X	X
180 (1 PM)	X		X	X		X	X	X				X
200	X	X	X	X	X	X	X	X	X	X	X	
205		X			X							
210		X			X							
220	X		X	X		X	X	X				X
225	X		X	X		X						
230	X		X	X		X						
235	X		X	X		X						
240 (2 PM)	X		X	X		X	X	X	X	X	X	X
Number	20		20	20		20	15	15	10	10	10	11
Volume (ml)	10	3	84	10	3	80	60	30	20	20	40	44

Total blood loss = 404 ml

ORAL GLUCOSE TOLERANCE TEST (OGTT)

TIME (min)	Glucose (0.5 ml)	Insulin (2 ml)	C-peptide (2 ml)	Glucagon (2 ml)
-30	X	X	X	X
-15	X	X	X	X
0	X	X	X	X
30	X	X	X	X
60	X	X	X	X
90	X	X	X	X
120	X	X	X	X
Number	7	7	7	7
Volume (ml)	3.5	14	14	14

Total blood volume = 45.5 ml