Storage disease on Autopsy

HOSP #		WARD	Histopathology
CONSULTANT	Dr. Jody Rusch	DOB/AGE	1 week Female neonate

Abnormal Result

An email from a colleague and mentor summarizes the abnormal result the best:

From: Jody Rusch jody.rusch@nhls.ac.za
Date: 2020/05/27 14:26 (GMT+02:00)

Dear S

I am not sure if this will be an easy one to nail down without extensive testing or luck.

To summarise:

Female neonate
No mention of ethnicity
C-section – hydropic on US
Birth weight 2935 g

Low Apgars (2 and 5)
Intubated, ventilated, ICU
Did not grow
Demised on day 7 of life in ICU

Non-immune heart failure plus storage disorder:
IMDs can cause heart failure.
Most likely lysosomal storage disease (14 different ones have been associated with HF)
Most LSDs are AR inheritance
HF, facial dysmorphism, AR inheritance, previous sibling hx
Common in European populations (and it appears globally) include – Mucopolysaccharidosis type VII, Gaucher's disease,

and GM1-gangliosidosis
In SA: Gaucher’s disease (Ashkenazi-Jewish population) – GD2 should be considered in severe perinatal with HF

Extensive list of Lysosomal storage diseases associated with heart failure:
Gaucher disease, type II, Morquio disease, Hurler syndrome, Sly syndrome, Farber disease, GM1 gangliosidosis, I-cell disease, Niemann-Pick disease type A and type C, Infantile Sialic Acid Storage disorder, alpha-neuroaminidase deficiency, multiple sulfatase deficiency, and Wolman disease.

Consider also non-lysosomal diseases
Other IMDs:
Type IV (Anderson disease)
Congenital disorders of glycosylation
Zellweger syndrome
LCHAD
Primary carnitine defic
Smith Lemli Opitz Syndrome

Also hypothyroidism

If a specific diagnosis (beyond likely LSD) is required, and will be paid for, perhaps Invitae have a panel?
Hopefully Prof can weigh in on this and help guide further testing.

Kind regards
J

Presenting Complaint

The histopathologist contacted me regarding any “screening tests” for lysosomal storage diseases

History

Maternal hx:
39 yr old
Booked – normal bloods
35 weeks gestation
Previous pregnancy – stillbirth due to hydrops foetalis (normal karyotype)
No mention of consanguinity

Examination

Post mortem:
Eyes wide set
Left Ear malformed
Flattened nasal bridge
Hydrops foetalis (HF)
Steatosis – lung, liver, heart, placenta

Laboratory Investigations

Not available

Other Investigations

Not available

Final Diagnosis

Unknown

Take Home Message

Message from Prof David Marais:

Hi S & J

Interesting and I wish we could devote much more effort to solve these cases. Especially since this is the second time this mother has had this sad experience and the next pregnancy may result in the same.

On first principles:

1. This appears autosomal recessive
2. The dysmorphology eliminates many “simpler” inherited errors as homeostasis through the placenta settles imbalances. However, errors involving tissue differentiation, structural components may have dysmorphology. E.g. sterol synthetic defects, mucopolysaccharidoses…It is easy to exclude Smith Lemli Opitz with 1mL serum or plasma even at this stage. However, syndactyly is a very strong feature and hydrops is uncommon but described. Happy to do this if sample is available. Mt abn has been described as well and might explain steatosis though not likely.
3. Microvesicular steatosis in several organs is suggestive of incomplete mobilisation of FA into mitochondria for oxidation or inadequate oxidation in mitochondria. These disorders do not usually result in dysmorphology and it is said renal steatosis is typical. LCHAD deficiency has caused hydrops but not dysmorphism to the best of my knowledge. Wolman’s disease has adrenal calcification but not hypoplasia as far as I know and not typically hydrops and diffuse steatosis – will need to check this again. The steatosis could be secondary to severe metabolic stress.
4. Hydrops fetalis should be taken as a strong clue. The lysosomal disorders can cause these. The list I found in JIMD Reports (2018) Hurler syndrome (MPS-I; OMIM #607014), Morquio-A (MPSIVA; OMIM #253000), Sly syndrome (MPS-VII; OMIM #253220), galactosialidosis (OMIM #256540), sialidosis
(OMIM #256550), GM1 gangliosidosis (OMIM #230500),

Gaucher type 2 (OMIM #230900), Niemann-Pick disease types A and C (NPD-A and NPC; OMIM #257200, 257220), Farber granulomatosis (OMIM #228000), Wolman disease (OMIM #278000), mucolipidosis II (I-cell disease; OMIM #252500), sialic acid storage disease (ISSD; OMIM #269920), and multiple sulfatase deficiency (OMIM #272200) which have been shown to be associated.

5. Interesting that the spleen is absent and that the adrenals are small. This is hard to explain on any of the metabolic disorders above but I shall have to read more extensively.
6. It may be worth testing the urine or other fluids for sialic acid. Infantile Salla disease is a possibility. (Sialin defect, coarse facies, hydrops fetalis, vacuolated lymphocytes but I was not aware of steatosis.) I can look up if this is practicable with the old fashioned assays and chemicals that we do have. I know I tried to analyse sialic acid in the early 1990s. Will look this up too.

One hopes that the anat path dept keeps samples for work-up. Very important to involve the chem path early if any metabolic disease is suspected as one can do fibroblast biopsy up to a few days in the morgue.

Regards
D

Professor Emeritus AD Marais
Chemical Pathology 6.33 Falmouth Building
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Anzio Rd, Observatory, 7925
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A case of hypertriglyceridemia with Diabetes mellitus

HOSP #		WARD	Albow Gardens Clinic
CONSULTANT	Prof. David Marais	DOB/AGE	31 y Male

Abnormal Result

31 y/o Male

Presenting Complaint

Triglycerides of 78.59 mmol/L

Lipaemia index 3 (value of 1132)

It is likely that the results as set out above was due to a routine follow-up, but unfortunately little clinical information was given by the clinician.

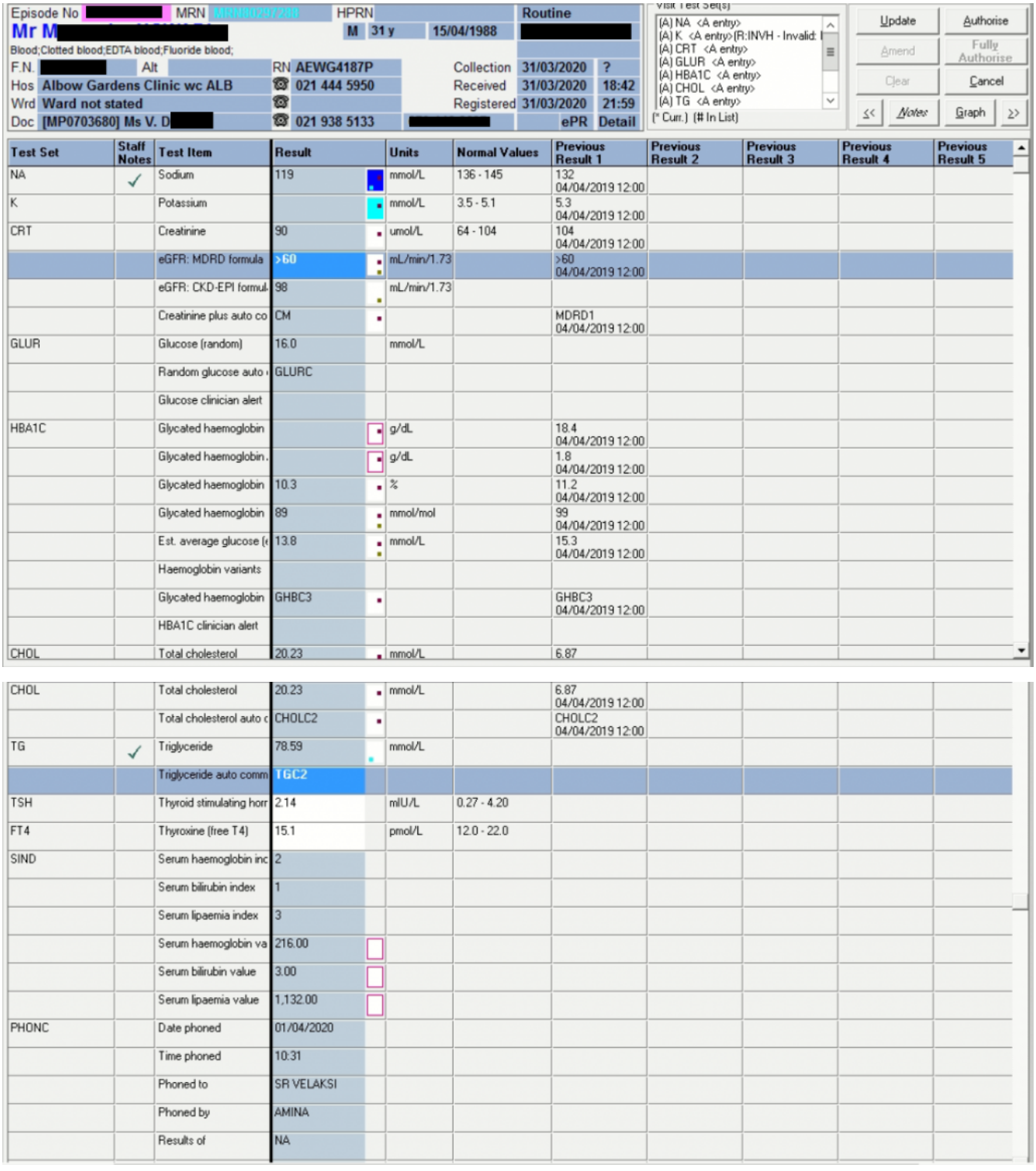
History

The patient is hypertensive and diabetic on treatment since 2018. No other clinical information was given and the drug list was not supplied.

Examination

N/A – No signs and symptoms obtained.

Laboratory Investigations



Episode No [redacted] MRN [redacted] HPRN Routine

Mr M [redacted] M 31 y 15/04/1988 [redacted]

Blood;Clotted blood;EDTA blood;Fluoride blood;

F.N. [redacted] Alt RN AEWG4187P Collection 31/03/2020 ?

Hos Albow Gardens Clinic wc ALB ☎ 021 444 5950 Received 31/03/2020 18:42

Wrd Ward not stated ☎ Registered 31/03/2020 21:59

Doc [MP0703680] Ms V. D [redacted] ☎ 021 938 5133 [redacted] ePR Detail

Visit Test Set(s)

(A) NA <A entry>
(A) K <A entry> (R:INVH - Invalid:
(A) CRT <A entry>
(A) GLUR <A entry>
(A) HBA1C <A entry>
(A) CHOL <A entry>
(A) TG <A entry>
(* Curr.) (# In List)

Update | Authorise | Amend | Fully Authorise | Clear | Cancel | << | Notes | Graph | >>

Test Set	Staff Notes	Test Item	Result	Units	Normal Values	Previous Result 1	Previous Result 2	Previous Result 3	Previous Result 4	Previous Result 5
NA	✓	Sodium	119	mmol/L	136 - 145	132 04/04/2019 12:00				
K		Potassium		mmol/L	3.5 - 5.1	5.3 04/04/2019 12:00				
CRT		Creatinine	90	umol/L	64 - 104	104 04/04/2019 12:00				
		eGFR: MDRD formula	>60	mL/min/1.73		>60 04/04/2019 12:00				
		eGFR: CKD-EPI formul	98	mL/min/1.73						
		Creatinine plus auto co	CM			MDRD1 04/04/2019 12:00				
GLUR		Glucose (random)	16.0	mmol/L						
		Random glucose auto	GLURC							
		Glucose clinician alert								
HBA1C		Glycated haemoglobin		g/dL		18.4 04/04/2019 12:00				
		Glycated haemoglobin		g/dL		1.8 04/04/2019 12:00				
		Glycated haemoglobin	10.3	%		11.2 04/04/2019 12:00				
		Glycated haemoglobin	89	mmol/mol		99 04/04/2019 12:00				
		Est. average glucose	13.8	mmol/L		15.3 04/04/2019 12:00				
		Haemoglobin variants								
		Glycated haemoglobin	GHBC3			GHBC3 04/04/2019 12:00				
		HBA1C clinician alert								
CHOL		Total cholesterol	20.23	mmol/L		6.87				
		Total cholesterol	20.23	mmol/L		6.87 04/04/2019 12:00				
		Total cholesterol auto	CHOLC2			CHOLC2 04/04/2019 12:00				
TG	✓	Triglyceride	78.59	mmol/L						
		Triglyceride auto comm	TGC2							
TSH		Thyroid stimulating horr	2.14	mIU/L	0.27 - 4.20					
FT4		Thyroxine (free T4)	15.1	pmol/L	12.0 - 22.0					
SIND		Serum haemoglobin inc	2							
		Serum bilirubin index	1							
		Serum lipaemia index	3							
		Serum haemoglobin va	216.00							
		Serum bilirubin value	3.00							
		Serum lipaemia value	1,132.00							
PHONC		Date phoned	01/04/2020							
		Time phoned	10:31							
		Phoned to	SR VELAKSI							
		Phoned by	AMINA							
		Results of	NA							

Other Investigations

We would have loved to do lipid electrophoresis and see better investigations into the cause of the diabetes, but at the time of writing, 14/05/2020, the patient has unfortunately not had

the opportunity to follow-up and it can unfortunately not be shown.

In an adult diabetic one would however expect the lipid electrophoresis to be that of a Fredrickson type V.

Hyperlipoproteinemia type V, also known as mixed hyperlipoproteinemia, familial or mixed hyperlipidemia, is very similar to type I, but with high VLDL in addition to chylomicrons.

It is also associated with glucose intolerance and hyperuricemia.

Final Diagnosis

Considering most factors known, and as explained via feedback from Prof. Marais below, diabetes is likely type 2 related to insulin resistance. One should also consider metabolic errors such as glucokinase deficiency causing MODY. Other causes, but unlikely, are endocrine pancreatic insufficiency which could include mitochondrial defects or herbicide-induced diabetes or (post-traumatic) excision of tail of pancreas. HbA1c shows prolonged exposure to high glucose concentrations: 10.3% and 11.2% the year before.

Take Home Message

The following were my thoughts on causality of the high triglycerides initially:

Increased intake:

- Overeating (unlikely for this high Triglyceride level), that is why one has lipoproteins – to keep the fat in the blood low and store the fat in liver and tissues.
- Excess alcohol consumption, but I'm also not sure

(wasn't sure) if excess alcohol will raise triglycerides this high – it likely may (after prof's email I think this is very likely the main cause in this patient).

Increased production:

- Kidney failure (nephrotic syndrome) (Prot: Creat ratio will likely exclude this – if borderline, a protein electrophoresis can be done).

Decreased metabolism:

- Some forms of familial hyperlipidemia such as familial combined hyperlipidemia
- Lipoprotein lipase deficiency
- Lysosomal acid lipase deficiency (aka cholesteryl ester storage disease)
- Hypothyroidism (TFT's normal in this patient though)
- SLE
- Glycogen storage disease type 1 –> NAFLD (non-alchoholic fatty liver disease)

Drugs:

- Isotretinoin, Thiazides,
- Apparently some HIV meds.

How to further test:

Lipid electrophoresis will delineate the Fredericksen Class.

Familial combined hyperlipidemia:

Lipid electrophoresis will show lipoproteinemia type IIB.

LPL deficiency:

Lab tests show massive accumulation of chylomicrons in the plasma and corresponding severe hypertriglyceridemia. Typically, the plasma in a fasting blood sample appears creamy (plasma lactescence).

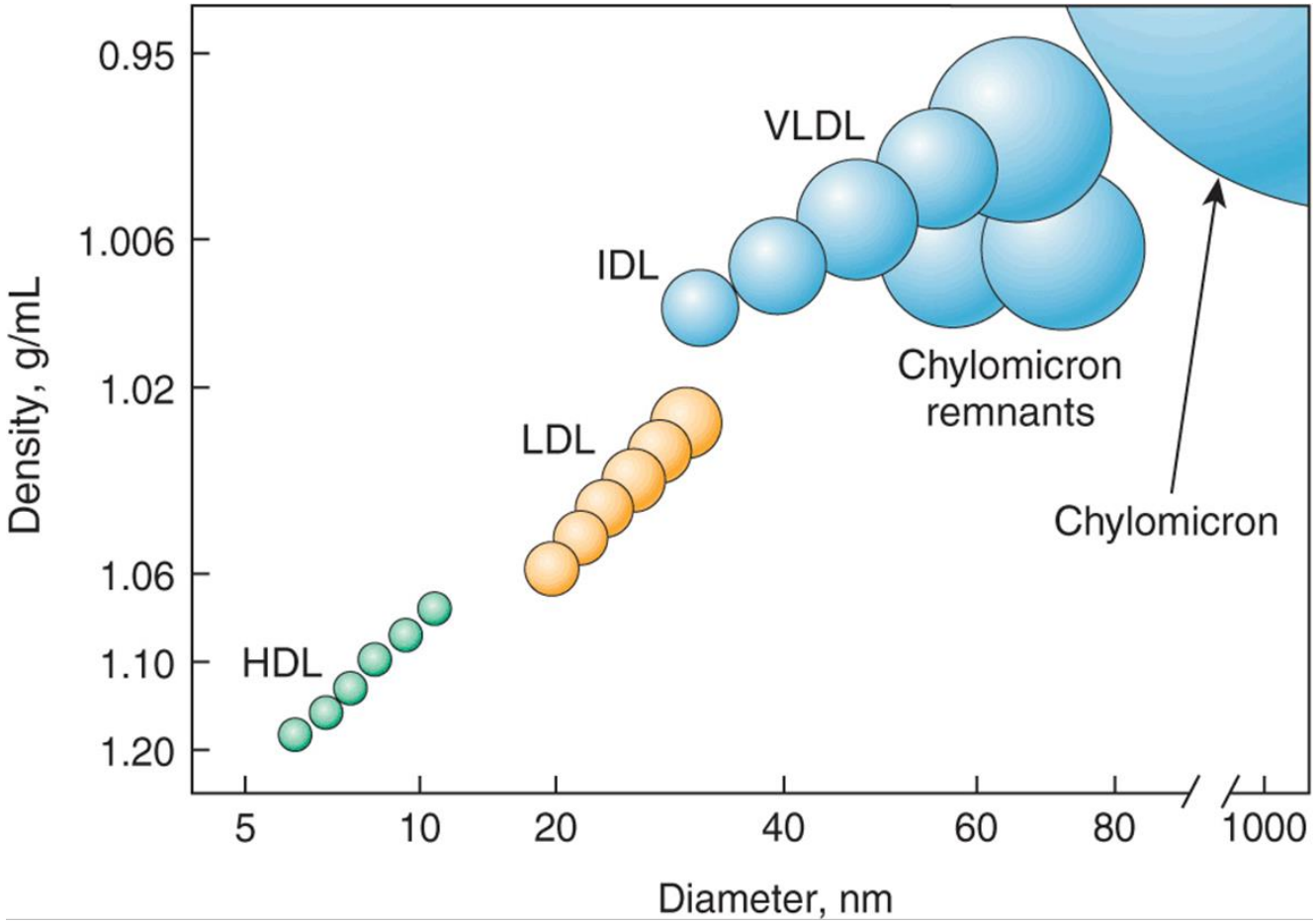
The absence of secondary causes of severe hypertriglyceridemia (like e.g. diabetes, alcohol, estrogen-, glucocorticoid-, antidepressant- or isotretinoin-therapy, certain antihypertensive agents, and paraproteinemic disorders) increases the possibility of LPL deficiency. Also other loss-of-function mutations in genes that regulate catabolism of triglyceride-rich lipoproteins (like e.g. ApoC2, ApoA5, LMF-1, GPIHBP-1 and GPD1) should also be considered. (remember our case – I won't mention her name though, patient's name begins with a K… and ends with …ana).

The diagnosis of familial LPL deficiency is finally confirmed by detection of either homozygous or compound heterozygous pathogenic gene variants in LPL with either low or absent lipoprotein lipase enzyme activity (Jody and I have done this assay with Bharati and Prof once for above patient).

Lysosomal acid lipase deficiency (LAL-D) (aka cholesteryl ester storage disease) – Unlikely – would rather present earlier – the accumulation of fat in the walls of the gut in early onset disease leads to serious digestive problems including malabsorption, the gut fails to absorb nutrients and calories from food. Because of these digestive complications, affected infants usually fail to grow and presents with failure to thrive. As the disease progresses, it can cause life-threatening liver dysfunction or liver failure). Until 2015, apparently there was no treatment (not sure if this is true though), and very few infants with LAL-D survived beyond the first year of life.

I think the clinical presentation and examination and history is much needed before any further investigations are advised.

Also, one should appreciate the size difference which is partly responsible for the electrophoretic mobility of lipoproteins on a gel.



Lipoprotein size illustration

Feedback from Prof. David Marais:

Hi Dieter

Thanks for distributing the interesting case information. The patient is at very high risk of developing acute pancreatitis. Hopefully the medical officer will be able to get in touch with the patient and urgently:

- (1) control diabetes mellitus and
- (2) restrict dietary fat intake to 10g/d for a few days whereafter 30-40g/d.
- (3) restrict alcohol intake to preferably zero or certainly <20g/d.
- (4) prescribe fibrate.
- (5) Referral to the lipid clinic – unfortunately may take time owing to shut-down of out-patients clinics in the precautions against corona virus spread.

Such severe hyperTGaemia seen in the neonate, infant or child is most likely due to an error in the lipolytic system and all of these are recessively inherited. LPL deficiency is the commonest but there may also be apoCii, apoAv, GPIHBP1 or LMF1 deficiency. In adolescent and young adults the same causes apply but also auto-immune LPL inhibition. In these cases all the agarose gel electrophoresis for lipoprotein separation will display a type I pattern. The highest TG conc I have seen in a patient was 695mmol/L and at 6 weeks of age.

In adults the lipoprotein electrophoresis pattern will usually be a type V. Here, there is usually partial lipase deficiency (often polygenic heterozygotes of LPL system components) and a dietary or metabolic stress. Diet containing triglycerides in large amounts and alcohol. Metabolic stress is mostly diabetes with increased return of NEFA to the liver and export as VLDL. Occasionally, apoE2/2 status with impaired remnant clearance can have a backlogue effect to raise VLDL and chylomicrons. Rarely, in partial lipodystrophies the adipose tissue does not take up NEFA from LPL and the liver puts out more VLDL which competes with chylomicrons for lipolysis. Typically this is associated with diabetes as well. Significant hypothyroidism and renal impairment appear to be excluded as potential secondary causes.

The results indicate long-standing diabetes and hyperlipidaemia. There is likely pseudohyponatraemia. This is because the aqueous part of the aliquot for analysis can be significantly less than the whole volume. The response is to do highspeed or ultracentrifugation so that the lipid can float and the infranatant plasma can be best analysed. Obviously, the whole plasma should be first assayed (in dilution with saline) to be certain of the TG concentration. Alternatively, the lipid volume can be calculated by converting the mmol/L of TG + CE + phospholipid to mass/L and then using the specific gravity of 0.92 g/mL to obtain the volume correction. For practical purposes only the TG and

cholesterol values may be used as we do not routinely measure the phosphatidyl choline. Average MW of TG =850da, of CE = 650da, of PL = 750da. Note that usually 70% of cholesterol is esterified. Cholesterol MW = 387da.

Per L, TG of 79mmol/L is 67g, CE of 14mmol/L is 9g, total lipid is 85g.

Each g being 1.09mL, makes this 92.4g/L or 9.24g/100mL. This means that the aqueous portion of the aliquot is about 10% too low. This makes the calculated Na+ concentration about 131mmol/L which is still not normal but certainly closer to the reference range. But the calculation is not highly accurate; partly because PL has not been taken into account; unesterified cholesterol is quantitatively less important.

Diabetes is likely type 2 related to insulin resistance but at this age and especially if dominantly inherited, consider metabolic errors in MODY such as glucokinase deficiency. Unlikely endocrine pancreatic insufficiency which could include mitochondrial defects or herbicide-induced diabetes or (post-traumatic) excision of tail of pancreas. Not certain if patient is on IV line that could provide lipid (Intralipid in parenteral nutrition) or glucose. Regardless, HbAic shows prolonged exposure to high glucose concentrations.

Regards

D

Professor Emeritus AD Marais

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