Author's response to reviews

Title: Lipoprotein lipase activity and mass, apolipoprotein C-II mass and polymorphisms of apolipoproteins E and A5 in subjects with prior acute hypertriglyceridaemic pancreatitis

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Author's response to reviews: see over
To the editor BMC Gastroenterology:

Here is our point-by-point reply to the referees. We would like to thank the Editor and Referees, giving us the opportunity to improve our manuscript. In this cover-letter and in the new version of our manuscript, modifications from the original are in red, in order to make reading easier.

On behalf the authors,

Pedro Valdivielso

Reviewer's report
Title: Lipoprotein lipase activity and mass, apolipoprotein C-II mass and polymorphisms of apolipoproteins E and A5 in subjects with prior acute hypertriglyceridaemic pancreatitis
Version: 1 Date: 26 March 2009
Reviewer: John Brunzell

Reviewer's report:

Major:
1. The authors addressed whether biochemical or clinical differences could be noted in severely hypertriglyceridemic patients who had and did not have pancreatitis. Other than finding 5 patients with LPL deficiency, there were few differences. A borderline increase in apoA5 snp was noted in HTG with pancreatitis. The first paragraph is not clear about this. We have added a short sentence in the 1st paragraph in discussion emphasizing this finding.

2. Results: Estimates of positive family history are probably underestimates unless relatives were actually studied. This should be noted. We have added this limitation to our results.

3. The measures of chylomicron lipids are estimates at best. This technique is not able to distinguish between smaller chylomicrons and larger VLDL. Did they measure apoB48 and apoB100? NMR has not been shown to differentiate between these particles either. They should call their chylomicrons "estimated". We completely agree with the referee; due to this we already called our chylomys chylomicron-like particles (see text and figure 1). We have added a short sentence indicating this fact.

Minor:
1. Page 3, line 2: "1.3-3.5%" of what? This is the frequency of hypertriglyceridaemic pancreatitis among patients with acute pancreatitis. We've changed the sentence in order to clarify it.
2. Page 4, line 6: Delete word "promising". This is still experimental. Deleted.
4. Page 6, para 3: Split last sentence with period after word "plasma". Start second part of sentence with "One mU". We have corrected the sentence in the way suggested by the referee.
5. Results: Were fibrates started after the bouts of pancreatitis? This might lead to further underestimation of triglyceride levels. Absolutely. Most patient started therapy after the first episode of acute pancreatitis, leading to lower levels of Tg. Interestingly, both groups (HTG and non-deficient patients with AP) were well balanced in terms of fibrate therapy.
6. Put BMI into table, perhaps in place of weight. We have replaced body weight and height by BMI in the table.
7. Page 10, end of para 2: add "or by measuring postheparin plasma LPL activity". We have added the sentence.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.

Reviewer's report

Title: Lipoprotein lipase activity and mass, apolipoprotein C-II mass and polymorphisms of apolipoproteins E and A5 in subjects with prior acute hypertriglyceridaemic pancreatitis
Version: 1 Date: 3 April 2009
Reviewer: Emilio Ros
Reviewer's report:

This paper compares a group of patients with past hyperlipidemic acute pancreatitis (AP) with a group of patients with similar triglyceride elevations but no history of AP for clinical features, lipoprotein and apolipoprotein levels, LPL mass and activity, apoE genotype and a relevant apoA5 polymorphism. There are not many relatively large series of hyperlipidemic AP in the literature, and the biochemical and genetic studies performed here are important. The main message is that severe LPL deficiency is not a common cause of hyperlipidemic AP, but is still identifiable in a substantial minority of patients.

Major compulsory revisions
1.- The series seems is biased towards male patients with moderate alcohol drinking and might include cases of alcoholic pancreatitis. Alcohol consumption is critical, as some “moderate drinkers” (what does it mean?) may go on a week-end binge and develop pancreatitis together with elevated triglycerides, but such an episode would be called “alcoholic AP”, not “hypertriglyceridemic AP”. Authors must make sure that there were no patients with alcoholic pancreatitis in their series, no matter how elevated triglycerides were at admission. The fact that there were many men and few women in these series argues in favour of an alcoholic etiology for HTG. Please, re-evaluate the clinical histories or the own patients to attempt to find out daily grams of alcohol consumed at the time of AP. That's a very important issue. We considered moderate drinkers those individuals consuming < 40 g of alcohol by day (men) or < 20 g/day by women. No one in our series was heavy drinker. On the other hand, in most series of hypertriglyceridemia, 3 out of 4 patients are men; in cases of severe HTG (ie those having Tg above 1000 mg/dL) the frequency of men are even higher, approaching 90%; thus, it is not rare the clear predominance of men in both groups. We have added in the text a clear definition of alcohol consumption.

2. Many patients were investigated while under fibrate treatment, with ensuing modification of lipoprotein values and LPL activity. We agree with the referee, and fibrate therapy increases LPL activity; in fact, in our patients we found fibrate therapy as an independent factor associated to LPL activity (data not shown). However, it should be noticed that both groups were treated with fibrates in the same extent and so it was not responsible for differences in both groups.

3. Table 1 shows that nearly 50% of patients in both groups were under fibrate treatment? If these patients had chylomicronemia syndrome, how were the remaining 50% treated? Were they receiving marine n-3 fatty acids and at what doses? Else, in the case of the patients with prior AP, had some of them lost so much weight since the AP episode that they were now nearly normolipidemic and did not require treatment? Data on weight changes from admission for AP to present evaluation would be interesting to know. All patients were counseled to follow diet and exercise as a main therapy, avoiding alcohol. Those having LPL deficiency were under a low-fat diet, assuming there is no effective drug for this condition. Few patients were receiving low doses of omega-3 fatty acids, mainly < 1.0 g/day (our study was initiated when there was no commercially available prescription omega 3 fatty acids in our country). We have expanded the information about drug therapy in table 1.
Minor essential revisions
M & M:
1. HP is not a good acronym. Suggest using HTG with AP and HTG without AP to describe the 2 groups. Respectfully, we prefer HP (hypertriglyceridaemic pancreatitis) that the proposed by the referee; HTG with AP can be interpreted by the reader as HTG “with any kind of AP”, which is not the case in our paper.

2. The methods for biochemical analyses are well described, except lipoprotein separation. Please, describe with more detail (ie, densities for each lipoprotein class). A more detailed description of lipoprotein separation has been added to the text.

3. There is no clear description of the study protocol, i.e., the information sought from the patients, measurement of anthropometric variables and adiposity, how was family history of dyslipidemia ascertained: Were all available relatives of each patient screened or the information was based on patients’ recall?. All information from patients, including anthropometry, was taken again the same day the postheparin blood sample was obtained for LPL measuring. What were the diagnoses, i.e, familial hypertriglyceridemia (FHTG), familial combined hyperlipidemia, etc.? FHTG is a likely disorder in patients with chylomicronemia. Which diagnostic criteria were used for the familial lipid disorder? AF is not a good acronym; why not use “Familial hyperlipidemia”? Family history was obtained on the first visit to the Lipid Clinic; for most patients, relatives were not available, thus most of the information relies on patient’s recalls. Due to this, we were unable to give reliable information for the etiology of primary hypertriglyceridemias. Alcohol consumption is critical, as stated above. See above. A complete list of medications used should be listed. A complete list of medication has been added to table 1, as already mentioned. Also, given that Table 1 lists fatty liver as comorbidity, was a liver ultrasound obtained in each patient and only those shown in Table had sonographic criteria for fatty liver? All this should be explained in a paragraph or two dealing with the study protocol. US of liver was available for all patients with HTG and AP; US of the liver is performed at our Lipid Clinic if patients show high transaminases or γGT. In other words, those subjects with HTG and no fatty liver were at list with no analytical and/or ultrasonographic criteria for fatty liver.

Results:
4. Review of histories from admission for AP. The lipid profile nearest the episode of AP and the cholesterol and triglyceride values should be shown in Table 2 as “admission lipid values” in medians (interquartile ranges) for those with such data. Most data from patients with lipaemic pancreatitis around the episode were taken many hours after they were diagnosed and admitted to the hospital (sometimes even more than 24 hours). Occasionally, as stated in M & M, they were considered as HTG with HP if serum at admission was grossly
lipaemic. Due to this, we were unable to show confident data nearest the episode as suggested by the reviewer.

5. Table 2. There must be data errors, as chylo cholesterol is too high (chylos contain very little cholesterol compared to Tg) and chylo Tg is higher than total Tg in the AP series (?). There are no data errors; as stated in table 2, mean ± SD for cholesterol and Tg in Chylos are shown only for those who have Chylos (N = 32); by contrast, total Tg in table 2 comprises for all patients included in the study, having or not chylos at fasting (N = 55). One wonders about the precision of the chylo isolation method used (contamination with other lipoprotein fractions?), as data from Figure 1 and statements about chylos being present in 12 fasting samples with Tg below 5.65 mmol/L are puzzling (one does not see 12 points below this level in Figure 1). Ultracentrifugation as we used in our study is a common procedure to measure chylomicrons, but it is not free of small contamination from VLDL (richer in cholesterol than chylos); however, in our patients, the cholesterol/Tg ratio in Chylos were 0.2 ± 0.06, which is really close to the normal ratio found in isolated human chylomicrons. Furthermore, we agree with the referee on the difficulty identifying 12 points below 5.56 mmol/L; the reason is the scale of the graph; please, note highest values are over 40 mmol/L, thus many points are superimposed. See below what happens if the higher value is 5.65 mmol/L. That values are expressed in mmol/L should be shown in Table 2. We added units at the bottom of table 2. As usual, Tg data are skewed (SD higher than mean), thus should be shown as medians (IQ ranges). We show Tg as median and IQ ranges in tables. ApoE genotypes (with all alleles, not only 33 and no33) and the tested apoA5 polymorphism should be shown in this Table (with number preceding percentage). We show data in table 2 as suggested by the referee.

6. Figure 1. See above for the not obvious 12 data points below 5.65 mmol/L.
7. Table 3. Should be omitted, as it repeats data (with lower numbers) in the first two columns. Explain in text distinguishing clinical features of patients with LPL deficiency. According to the first referee, we have modified that table.

8. Table 4. Comparisons of lipid/lipoprotein/apolipoprotein values among the 3 groups in this Table have little meaning because all 5 patients with LPL deficiency were untreated while most of the other patients were under fibrate treatment, which may also increase LPL activity. To be fair, authors should compare lipid values among untreated patients in each group. Why were the LPL deficient patients untreated while having such high triglyceride levels?. We don’t agree with the referee. First of all, it is clear that only patients who were deficient for LPL had different figures for many parameters; in other words, non-deficient patients with severe HTG are indistinguishable in clinical and analytical terms. Secondly, by chance, both groups of non-deficient HTG were balanced in terms of fibrate treatment, so we believe that our data will be the same comparing treated and untreated patients. Thirdly, LPL deficient patients were treated with very low fat diet in order to keep Tg below 11.29 mmol/L, avoiding episodes of abdominal pain and AP. So far, there is no drug indicated for the therapy of LPL deficient patients.

9. Figure 2. The order of groups should be the same than in Tables (first HTG with PA). We have changed the order in the figure.

Discussion:
10. Limitations of the study should be acknowledged. An important limitation is that many patients in both groups were investigated while under treatment with
drugs (fibrates, perhaps fish oil) that profoundly affect the outcome measurements of lipid and lipoprotein levels and even LPL activity. We have added a sentence indicating the limitations of our study.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: 
I declare that I have no competing interests