# A dynamic $^1$H-MRS study on intramyocellular lipid accumulation in the infusion of lipids differing in the degrees of saturated fatty acid acyl chains

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**Introduction.** Excess availability of lipid in the form of obesity (1), elevated plasma FFA or triglycerides (2-3) have been correlated with insulin resistance. Interestingly, the quality as well as the quantity of fat influences the degree of insulin resistance: saturated fats > monounsaturates > polyunsaturates (4). Increased saturation of muscle membrane phospholipids correlates significantly with the degree of insulin resistance in cross sectional studies (5-6). Studies in humans have confirmed that short-term elevation of circulating free fatty acids and hyperinsulinemia leads to decreased insulin sensitivity (7-10); however some (7-8) but not all (9) studies confirm this is paralleled by increased IMCL stores. The plasma FFA derived from intravenous (i.v.) infusion of Intralipid or Liposyn triglyceride emulsion are highly unsaturated due to their origin as soybean or a mixture of soybean and safflower oils. No studies to date have examined the acute effects of enterally delivered fats varying in fatty acid saturation on the time course of induced insulin resistance. The aim of the present study was to 1) determine whether a lipid infusion rich in C18:1 will induce changes in IMCL; 2) compare the difference in the change of IMCL content under lipid infusions differing in the degree of saturation of the fatty acid acyl chains; 3) study the effect of lipid on insulin sensitivity.

**Methods.** ***Subjects***: Subjects with normal glucose tolerance (1F/8M) (BMI: 24.9±1.2 kg/m$^2$, range: 20.3 ~ 29.9 kg/m$^2$. Age: 29.8±2.4 yr, range: 21 ~ 42 yr) were studied. 6 subjects participated in glycerol (G) control, 5 in triolein (TO), 2 in trilinolein (TL) and 1,3-dioleate-2-palmitate (DOMP) studies. 2 subjects participated in all 4 studies, i.e. G, TO, TL and DOMP. ***Euglycemic-hyperinsulinemic clamps***: Constant hyperinsulinemia was achieved by a continuous i.v. insulin infusion of 40mU/m$^2$/min. Plasma glucose was kept in the euglycemic range by a variable i.v. glucose infusion (20%). Lipid emulsion was infused through a nasal duodenal (n.d.) tube at a rate of 40mls(20%)/m$^2$/h. For the control study, glycerol was infused through the n.d. tube at a rate of 800mg/m$^2$/h. ***$^1$H-MRSI***: IMCL of right calf muscles in soleus (S) and tibialis anterior (TA) were measured by $^1$H-MR spectroscopic imaging ($^1$H-MRSI) (TR/TE=1000/24 ms) in a 4T Varian Inova whole body MR system with a TEM $^1$H resonator. In-plane phase encoding (32×32) over 16.0-×16.0 cm$^2$ with a 1-cm slice thickness resulted in nominal voxel resolution of 0.25 ml. IMCL was measured at times 0, 1, 2, 3, 4, and 5h. The absolute IMCL concentration was calculated using internal skeletal muscle tissue water, an 80% tissue water fraction and tissue density of 1.05 g/ml (11, 12). Methylene proton density was assumed to mimic triolein (61.0 mmol/ml) or was corrected for the proton density of the 3 lipid emulsions (11).

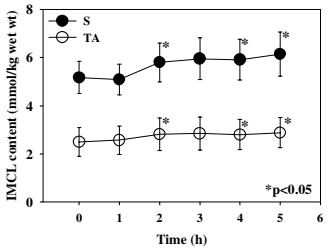


Fig.1 IMCL contents in S and TA with TO infusion.

**Results**. The IMCL contents (n=4) stayed unchanged in either muscle after 5h G infusion (4.8±1.2 vs. BL: 5.0±1.4 mmol/kg wet wt in S; 2.6±1.2 vs. BL: 2.9±1.4 mmol/kg wet wt in TA; both NS), whereas the TO infusion led to a steady increase in IMCL contents in both muscles. After 2h, IMCL contents were already significantly higher than that at baseline in both muscles as shown in Fig.1. In 5h TO infusion, the IMCL content (n=5) showed an elevation of 18% compared to the baseline (6.1±0.9 vs. BL: 5.2±0.7 mmol/kg wet wt, p=0.026) in S and 23% (2.9±0.6 vs. BL: 2.5±0.6 mmol/kg wet wt, p=0.011) in TA. Two male subjects participated in three lipid infusions, i.e. TL, TO and DOMP. The IMCL contents in S increased 11.8%, 23.9% and 35.7% after 5h infusions of TL, TO and DOMP, respectively (Fig. 2, black bar). Similar results were also seen in IMCL of TA (not shown here). The IMCL contents were initially estimated assuming 61.0 mmol of $^1$H/ml TG for all lipid infusion studies. The larger increment in IMCL content was also corrected for the differences in proton density of the component fatty acyl chains of the relevant lipid emulsions, i.e. 43.7, 61.0, 67.3 mmol/ml for TL, TO and DOMP, respectively (11). The IMCL increment, after normalization for TG proton density, was still significantly increased after infusion with lipid emulsions of greater degree of acyl chain saturation (Fig. 2, grey bar).

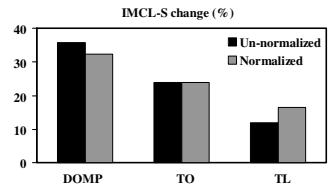


Fig.2 IMCL-S change (%) in 5h compared to BL value with different lipid infusions.

Fig. 3 shows the time course of the whole-body glucose disposal rate, Rd, with infusion of G and all lipids (TO, TL and DOMP). Rd under lipid infusion was significantly lower compared to the control study from 5h till the end of the study. The total FFA concentration at 330 min of lipid infusion was significantly higher than during G infusion (p=0.005) (Table 1). The baseline plasma was rich in C18:1 (~45%), followed by C16:0 (~25%), C18:2 (16%) and C18:0 (~14%). With the infusion of lipids differing in the fatty acid profile, the plasma at 330 min showed elevation of corresponding fatty acids introduced from the exogenous lipid, confirming their efficient absorption (Table 1).

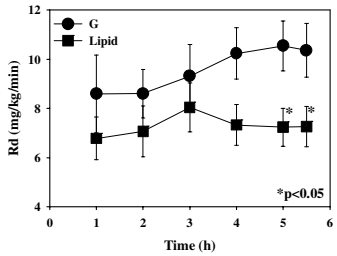


Fig.3 Rd with infusion of G and Lipid.

**Discussion and Conclusion.** IMCL content, in both S and TA muscles, started to rise within 2h of lipid infusion induced through an n.d. tube. The increment in IMCL content was also affected by the degree of saturation of the fatty acid acyl chains, even after normalization for differences in their methylene proton densities. This implies that saturated fats tend to be stored in preference to being oxidized for energy generation, which would potentially increase the risk of insulin resistance, obesity and associated metabolic disorders. The insulin sensitivity was impaired significantly in 5h lipid infusion compared to glycerol control study under insulin stimulation. This insulin resistance is caused, in part, by the elevated free fatty acid with induction of lipid through n.d. tube.

In conclusion, this study showed that IMCL content increased rapidly with enteral infusion of lipid under hyperinsulinemia and the increment of IMCL content was also manipulated by the degree of saturation of the fatty acid acyl chains. Short-term elevation of lipid availability reduced peripheral insulin sensitivity in humans with normal glucose tolerance.

Table 1. Plasma total FFA concentration ([FFA]) and fatty acid composition at 330 min.

| | G | TO | TL | DOMP |
|---|---|---|---|---|
| [FFA] ,μmol/l | 91.2 | 292.3 | 376.0 | 384.0 |
| C16:0, % | | 9.5 | 12.2 | 27.0 |
| C18:0, % | | 8.3 | 9.0 | 7.3 |
| C18:1, % | | 76.7 | 9.5 | 61.4 |
| C18:2, % | | 5.5 | 69.3 | 4.2 |

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