Effect of the Pattern of Elevated Free Fatty Acids on Insulin Sensitivity and Insulin Secretion in Healthy Humans

N. Stefan, H. G. Wahl, A. Fritsche, H. Häring, M. Stumvoll

Medizinische Klinik, Abteilung für Endokrinologie, Stoffwechsel und Pathobiochemie, Eberhard-Karls-Universität, Tübingen, Germany

In order to investigate whether the pattern of elevated free fatty acids (FFAs) has any effect on insulin sensitivity and insulin secretion in humans, we produced 2 distinct serum FFA patterns (PT 1 and 2) by infusing 6 healthy volunteers with 2 different lipid emulsions plus heparin for 24 hours. A hyperglycemic clamp (approx. 8 mM, 140 min) was performed before and 5 and 24 hours after both lipid infusions to determine insulin sensitivity and insulin secretion simultaneously. Total FFAs had increased comparably by 24 hours (2020 ± 268 μM in PT 1) and (1812 ± 154 μM in PT 2, $p = 0.24$). Serum PT 1 contained 66% saturated FFAs plus monoenes and 34% polyenes, while PT 2 contained 80% saturated FFAs plus monoenes and 20% polyenes. Thus, the ratio of polyunsaturated to saturated plus monoenes was about 0.5 in PT 1 vs. 0.25 in PT 2. In PT 1, the insulin sensitivity index (ISI) decreased by 20 ± 7% and 27 ± 10% from basal after 5 and 24 hours, respectively. In PT 2, the ISI decreased significantly more after 5 (41 ± 7%, $p = 0.008$) and 24 hours (52 ± 6%, $p = 0.005$). In contrast, different phases of insulin secretion did not change during the lipid infusion and did not vary between the two FFA profiles. In conclusion, these findings provide preliminary evidence that the composition of elevated serum FFAs influenced insulin sensitivity in humans. The FFA pattern low in polyunsaturated FFAs reduced insulin sensitivity more than the pattern high in polyunsaturated FFAs. In contrast, no effect on insulin secretion was observed.

Key words: Lipid Infusion – Gas Chromatography – Flame Ionization Detection – Insulin Action – Glycerol – Triglycerides

Abbreviations

FFA, free fatty acid; PT, pattern; ISI, insulin sensitivity index; ISR, insulin secretion rate; AIR, acute insulin response; TG, triglycerides.

Introduction

There is ample evidence for the important metabolic roles of free fatty acids (FFAs). They have long been recognized for their contribution to the development of peripheral insulin resistance. Interference with glucose uptake, glucose oxidation and glycogen synthesis in muscle is thought to be involved [1–3]. Moreover, elevated FFAs have been shown to increase endogenous glucose production [4], an effect secondary mainly to stimulation of gluconeogenesis [5]. On the other hand, experimentally raised FFA concentrations have been shown to stimulate insulin secretion in non-diabetic humans [6,7] and perfused rat pancreas [8].

In obesity, which is strongly associated with insulin resistance and increased risk of type 2 diabetes, the concentration and/or flux of FFAs is increased. The imbalance of the inhibitory FFA effect on insulin's peripheral (glucose disposal) and central (suppression of glucose production) action on the one hand and the stimulatory effect on insulin secretion on the other hand is thought to contribute to the pathogenesis of type 2 diabetes in obese individuals [4].

However, not only circulating levels of the FFAs *per se*, but also composition (chain length, degree of saturation) appear to mediate their metabolic effects. For example, a higher proportion of polyunsaturated serum FFAs in healthy men was associated with better insulin sensitivity [9]. Furthermore, in healthy humans, the degree of unsaturation of fatty acids (FA) found in phospholipids of skeletal muscle biopsies correlated negatively with fasting insulin [10]. This was interpreted as being the change in the FA composition of muscle modulating the action of insulin. In contrast, a 2-month dietary intervention with fish oil (rich in eicosapentaenoic and docosahexaenoic acid) vs. placebo failed to alter insulin sensitivity [11].

In addition to influencing peripheral insulin sensitivity, individual FAs have been shown to differentially affect insulin secretion *in vitro*. For the same total FFA concentration, for example, more saturated species (such as stearate) rendered the perfused rat pancreas far more responsive to glucose than the

Horm Metab Res 2001; 33: 432–438

ISSN 0018-5043

Received: 19 July 2000 Accepted after revision: 28 December 2000

polyunsaturated counterpart (for example, linoleate). Furthermore, the length of the carbon chain was positively correlated with glucose stimulated insulin secretion [12].

It is currently unknown whether the pattern of acutely elevated serum FFAs has an effect on insulin sensitivity in humans. Moreover, the influence of differences in FFA composition on insulin secretion has not been studied in humans. Therefore, we both raised total FFAs and produced 2 distinct serum FFA patterns (PT 1 and 2) by infusing 6 healthy volunteers with 2 different lipid emulsions plus heparin for 24 hours. We used the hyperglycemic clamp technique (approx. 8 mM, 140 min + arginine bolus) to assess insulin secretion and insulin sensitivity simultaneously before and 5 and 24 hours after both lipid infusions.

Methods

Subjects

After approval of the protocol by the local ethical committee and obtaining informed written consent from the trial subjects, we studied a control group (N = 6, 4 males, 2 females, age 29 ± 2 years, weight 68.3 ± 6.9 kg, BMI 21.8 ± 0.7 kg/m^2) who underwent a saline control protocol only and an experimental group (N = 6, 4 males, 2 females, age 28 ± 1 years, weight 74.0 ± 3.3 kg, BMI 22.2 ± 0.9 kg/m^2) who underwent 2 different lipid infusion protocols in random order approximately 1 week apart. Prior to the study, all subjects had their medical history taken and had taken a physical examination, a routine blood test and an ECG. Subjects were instructed to maintain their usual diet before the study.

Experimental protocols

For the experiments, subjects were admitted to the university's research unit at 6:00 AM after an overnight fast, and indwelling catheters were inserted into an antecubital vein for infusions and a dorsal hand vein was fitted in a retrograde fashion, which was kept in a heated chamber for arterialised blood sampling. In every protocol, baseline blood draws were performed at 6:45 AM (t-15 min) and at 6:55 AM (t-5 min).

During the entire study period, the subjects were supine and nil by mouth except for water.

The saline control protocol consisted of a baseline hyperglycemic clamp, followed by infusion of normal saline for 5 hours, followed by a second hyperglycemic clamp. The 2 lipid infusion protocols consisted of a baseline hyperglycemic clamp, followed by infusion of 2 different lipid emulsions MCT® or Omegaven® (Fresenius, Bad Homburg, Germany) at a rate of approx. 0.5 mg × kg^{-1} × min^{-1} plus heparin (bolus 1000 IE, 800 IE × min^{-1}) for 26 hours. A second hyperglycemic clamp was performed after 5 hours and a third hyperglycemic clamp after 24 hours. The lipid infusions were continued until the end of the third clamp. The FFA pattern generated by infusion of MCT was termed PT 1, that generated by Omegaven was termed PT 2. To suppress endogenous FFAs the subjects in the lipid protocols took acipimox (250 mg, Olbemox®, Pharmacia & Upjohn, Erlangen, Germany) every six hours during the 24 hours preceding the 2 lipid protocols and during the lipid infusions. This was to prevent dilution of "exogenous" FFA patterns by "endogenous" FFAs. Preliminary results had shown that indeed 2 distinct serum FFA patterns could be produced with these two commercially available lipid emulsions in addition to oral acipimox.

Hyperglycemic clamp

After baseline samples had been obtained, a 140 min hyperglycemic clamp was performed as previously described [13]. An intravenous bolus of 20% glucose over 1 minute was given to instantaneously raise blood glucose to 8 mM (bolus dose [mg] = body weight [kg] × desired increase in blood glucose [mg/dl] × 1.5). Subsequently, the glucose infusion was adjusted to maintain blood glucose at 8 mmol/l. At 120 min a bolus of 5 g, arginine was injected over 30 seconds.

The hyperglycemic clamp was chosen to assess insulin sensitivity and insulin secretion simultaneously. It had previously been shown that insulin sensitivity obtained from a hyperglycemic clamp is highly correlated with that obtained from a euglycemic clamp [14], and should therefore provide an adequate index for assessing changes in the same individual. For measurement of insulin secretion, the hyperglycemic clamp is considered to be the method of choice [13]. The glucose level of approx. 8 mM was chosen since effects of FFAs on insulin secretion were only seen at that level, but not at higher glucose concentrations [15]. Use of the arginine bolus as previously described [16] also allowed us to assess the effects of a non-glucose secretagogue.

Sampling and analytical procedures

During the hyperglycemic clamp, blood was sampled at t-15, – 5, 2.5, 5, 7.5, 10, 60, 80, 100, 120, 122.5, 125, 127.5, 130 and 140 min for determination of plasma insulin and C-peptide. Blood for determination of FFAs was sampled at t-15, – 5, 60 and 120 min of every hyperglycemic clamp and after 4, 11, 13 and 17 hours. Tubes were immediately placed on ice water. Serum insulin was determined by a microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan). Plasma C-peptide was determined by radioimmunoassay (Byk-Sangtec, Dietzenbach, Germany). Blood glucose was determined bedside every 2.5 to 5 min using a HemoCue analyzer (HemoCue, Mission Viejo, CA, USA).

The samples for FFA determination were spun down with minimal delay and frozen at – 20 °C until analysis. Total free fatty acid (FFA) concentrations were determined by an enzymatic method (NEFAC kit, WACO Chemicals, Neuss, Germany). Total lipids were extracted from serum and separated by thin layer chromatography (TLC) into individual classes. The FFAs were scraped off the TLC plate and transferred into screw-capped vials for direct transesterification [17]. The fatty acid methyl esters were quantified with cis-13,16,19-docosatrienoic acid as the internal standard using gas chromatography with a flame ionization detector (HP 5890, Hewlett-Packard, Waldbronn, Germany) on a 60 m × 0.25 mm × 0.2 µm fused silica column (Rtx 2330, Rystek, Germany). Helium was used as carrier gas at a column head pressure of 16 psi.

To illustrate the difference between the 2 patterns, we arbitrarily divided the fatty acids into polyunsaturated vs. saturated

plus monoenes (PU:SAT+M). This division produced the best separation between the 2 patterns.

Calculations and statistical analysis

The insulin secretion rate (ISR) was calculated as follows: Standard kinetic parameters for C-peptide (rate constants, volume of distribution) adjusted for age, sex, BMI and body surface area were used [18] and assumed to remain unchanged throughout the experiment. These parameters were used to calculate the ISR for the indicated intervals from the plasma C-peptide concentrations by deconvolution as previously described [19]. First-phase insulin release (1st PH) was considered to be the sum of the insulin secretion rates at 2.5 and 5.0 min in the hyperglycemic clamp experiment. Second-phase insulin release (2nd PH) was taken as the mean insulin secretion rate at 80, 100 and 120 min of the hyperglycemic clamp. The acute insulin secretory response to arginine (AIR) reflecting the increase above the prestimulus level was calculated as the ISR at 122.5 min minus 120 min.

Insulin sensitivity was assessed as an insulin sensitivity index (ISI), calculated by dividing the average glucose infusion rate during second phase insulin secretion (80 to 120 min) of the hyperglycemic clamp by the average plasma insulin concentration during the same interval.

Unless otherwise stated, the results are expressed as the mean ± SEM. Since the two lipid infusion protocols were performed in the same subjects, a paired Student's *t*-test (2-tailed) was used for statistical comparisons between the 2 FFA patterns. For comparison of changes over time, analysis of variance with the repeated measures design was performed. For comparison with the saline control protocol, the unpaired Student's *t*-test (2-tailed) was used. The statistical software package JMP (SAS Insitute Inc, Cary, NC, USA) was used.

Results

Free fatty acids-total concentration and profile

In the saline protocol total, FFAs increased from 471 ± 63 µM at baseline to 665 ± 100 µM after 5 hours ($p < 0.05$). During PT 1, total FFAs increased from 296 ± 100 µM at baseline to 1387 ± 311 µM before the clamp at 5 hours ($p < 0.05$), and to 1812 ± 154 µM before the clamp at 24 hours ($p < 0.01$). During PT 2, total FFAs increased from 236 ± 56 µM (p vs. PT 1 = 0.56) at baseline to 1491 ± 277 µM (p vs. PT 1 = 0.26) before the clamp at 5 hours ($p < 0.01$) and to 2020 ± 268 µM (p vs. PT 1 = 0.18) before the clamp at 24 hours ($p < 0.001$). In both protocols, FFAs fell by about 50% during each hyperglycemic clamp (Fig. **1**). The increase in both lipid protocols was greater than in the saline protocol (both $p < 0.01$).

The two serum FFA patterns are shown in Table **1**. PT 1 contained relatively more of linoleic and linolenic acid (ω3), while PT 2 contained relatively more of myristic, palmitic, palmitoleic and oleic acid. After 24 hours of lipid infusion, PT 1 contained 66 ± 1% saturated FFAs plus monoenes vs. 34 ± 1% polyunsaturated FFAs, while PT 2 contained 80 ± 1% saturated FFAs plus monoenes ($p < 0.05$ vs. PT 1) versus 20 ± 1% polyunsaturated ($p < 0.05$ vs. PT 1) ($p = 0.01$). This resulted in a ratio between polyunsaturated and saturated plus monoenes 0.5 in PT 1 and 0.25 in PT 2. The low standard errors (SEM) for the individual FFAs reflect the consistency of the profiles achieved in the individual subjects.

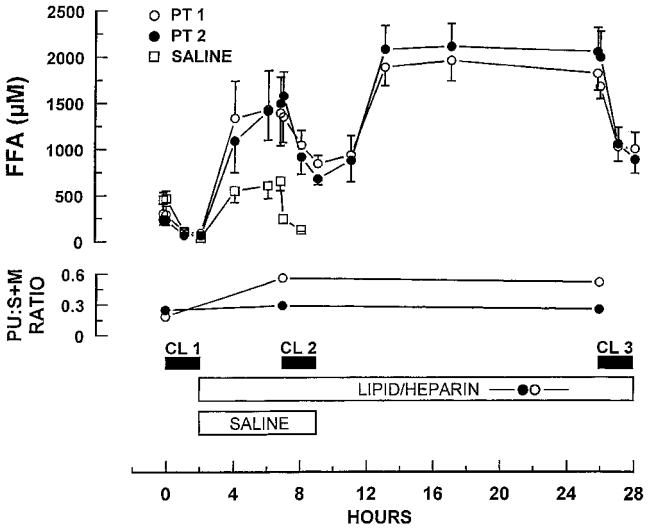

Fig. 1 Serum triglycerides, glycerol, total FFA levels and polyunsaturated: saturated plus monoenes (PU:S+M) ratio during the lipid and saline infusions. CL1, baseline clamp; CL2 clamp after 5 hours of lipid or saline infusion; CL3, clamp after 24 hours of lipid infusion.

Serum triglyceride (TG) concentrations

In the saline protocol, TG levels were 85 ± 14 mg/dl at baseline and 85 ± 15 mg/dl after 5 hours ($p = 0.98$). In PT 1, TGs decreased from 60 ± 13 mg/dl at baseline to 42 ± 6 mg/dl after 5 hours ($p = 0.15$) and increased to 62 ± 8 mg/dl after 24 hours ($p < 0.91$). In PT 2, TGs decreased from 61 ± 10 mg/dl (p vs. PT 1 = 0.93) at baseline to 53 ± 9 mg/dl (p vs. PT 1 = 0.36) after 5 hours ($p = 0.45$) and increased to 72 ± 15 mg/dl (p vs. PT 1 = 0.59) after 24 hours ($p = 0.42$).

Serum glycerol concentrations

In the saline protocol, glycerol concentrations increased from 52 ± 10 µM at baseline to 74 ± 11 µM after 5 hours ($p < 0.05$). In PT 1, glycerol levels increased from 59 ± 7 µM at baseline to 274 ± 32 µM after 5 hours ($p < 0.01$) and to 316 ± 23 µM after 24 hours ($p < 0.001$). In PT 2, glycerol levels increased similarly from 46 ± 5 µM (p vs. PT 1 = 0.17) at baseline to 241 ± 27 µM (p vs. PT 1 = 0.49) after 5 hours ($p < 0.01$) and to 354 ± 28 µM (p vs. PT 1 = 0.37) after 24 hours ($p < 0.001$). After 5 hours of lipid infusion, glycerol during the hyperglycemic clamp did not change significantly in PT 1 (from 274 ± 32 µM to 256 ± 37 µM [$p = 0.63$]), but did in PT 2 (from 241 ± 27 µM to 140 ± 10 µM [$p < 0.05$]). The decrease in PT 2 (–101 ± 25 µM) was significantly greater compared to PT 1 (–17 ± 31 µM [$p < 0.01$]).

After 24 hours of lipid infusion, glycerol during the hyperglycemic clamp did not change significantly in PT 1 (from 316 ± 23 µM to 291 ± 50 µM [$p = 0.56$]) but decreased in PT 2 (from 354 ± 28 µM to 201 ± 22 µM [$p < 0.01$]). The decrease in PT 2 (–153 ± 23 µM) was significantly greater compared to PT 1 (–25 ± 37 µM [$p < 0.05$]).

Table 1 Fatty acid profile of pattern 1 (PT 1) and 2 (PT 2)

Fatty Acid	PT 1 (% of total)			PT 2 (% of total)		
	pre basal	pre 5 hrs	pre 24 hrs	pre basal	pre 5 hrs	pre 24 hrs
Myristic (14:0)	3 ± 0	2 ± 0	1 ± 0	4 ± 0	4 ± 0	4 ± 0
Pentadecanoic (15:0)	2 ± 0	1 ± 0	1 ± 0	2 ± 0	1 ± 0	1 ± 0
Palmitic (16:0)	27 ± 1	21 ± 1	21 ± 1	23 ± 2	24 ± 0	25 ± 0
Palmitoleic (16:1 ω7)	1 ± 0	2 ± 1	3 ± 0	1 ± 0	6 ± 1	6 ± 0
Stearic (18:0)	22 ± 3	9 ± 1	8 ± 1	21 ± 1	7 ± 0	7 ± 0
Oleic (18:1 ω9)	24 ± 1	28 ± 2	30 ± 2	25 ± 0	33 ± 3	35 ± 1
Octadecanoic (18:1 ω7)	4 ± 1	2 ± 0	1 ± 0	5 ± 0	2 ± 1	2 ± 0
Linoleic (18:2 ω6)	10 ± 0	27 ± 1	27 ± 1	12 ± 0	10 ± 0	10 ± 0
Linolenic (18:3 ω6)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arachidic (20:0)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Linolenic (18:3 ω3)	2 ± 0	6 ± 0	5 ± 1	1 ± 0	2 ± 0	2 ± 0
Eicosatrienoic (20:3 ω6)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Behenic (22:0)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Arachidonic (20:4 ω6)	2 ± 0	1 ± 0	1 ± 0	2 ± 0	1 ± 0	1 ± 0
Eicosapentaenoic (20:5 ω3)	1 ± 0	0 ± 0	0 ± 0	1 ± 0	4 ± 1	3 ± 0
Docosatatraenoic (22:4 ω6)	1 ± 0	0 ± 0	0 ± 0	2 ± 0	0 ± 0	0 ± 0
Docosapentaenoic (22:5 ω3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0	0 ± 0
Docosahexaenoic (22:6 ω3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	4 ± 1	3 ± 1
Total	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Saturated	54 ± 2	33 ± 0	32 ± 1	50 ± 1	36 ± 0*	36 ± 0*
Unsaturated	46 ± 2	67 ± 0	68 ± 1	50 ± 1	64 ± 0*	64 ± 0*
Monoenes	29 ± 1	31 ± 2	35 ± 2	32 ± 0	41 ± 1*	44 ± 1*
Saturated + Monoenes	83 ± 1	64 ± 2	66 ± 1	81 ± 1	78 ± 2*	80 ± 1*
Polyunsaturated	17 ± 1	36 ± 2	34 ± 1	19 ± 1	22 ± 2*	20 ± 1*

The 2 pre-clamp samples were averaged, values are expressed as the mean ± SEM of 6 subjects. Please note that the absolute FFA levels differed markedly between the basal and the infusion state (see Results section). * p vs. PT 1 < 0.05.

Insulin sensitivity

During the saline control protocol, the ISI remained unchanged (0.035 ± 0.006 mg × kg^{-1} × min^{-1} × pM^{-1} at baseline and 0.033 ± 0.006 mg × kg^{-1} × min^{-1} × pM^{-1} after 5 hours, $p = 0.49$). During PT 1, the ISI decreased from 0.037 ± 0.007 mg × kg^{-1} × min^{-1} × pM^{-1} at baseline to 0.028 ± 0.005 mg × kg^{-1} × min^{-1} × pM^{-1} after 5 hours and to 0.025 ± 0.004 mg × kg^{-1} × min^{-1} × pM^{-1} after 24 hours. During PT 2, the ISI decreased from 0.040 ± 0.006 mg × kg^{-1} × min^{-1} × pM^{-1} at baseline to 0.023 ± 0.004 mg × kg^{-1} × min^{-1} × pM^{-1} after 5 hours, which was a greater decrease than that in PT 1 ($p = 0.008$ for the decrease). After 24 hours, the ISI decreased further to 0.018 ± 0.004 mg × kg^{-1} × min^{-1} × pM^{-1} in PT 2, which again was a greater decrease than in PT 1 ($p = 0.008$ for the decrease) (Fig. **2**, top panel). The relative decrease of the ISI from baseline was 20 ± 7% and 25 ± 10% after 5 hours and 24 hours, respectively, in PT 1 and 41 ± 7% ($p = 0.03$) and 52 ± 6% ($p = 0.005$) after 5 hours and 24 hours, respectively, in PT 2 (Fig. **2**, bottom panel). Analysis of variance yielded a p-value of 0.077 (pattern by time).

Insulin secretion

During the saline control protocol, 1st PH, 2nd PH and AIR of insulin secretion decreased slightly. Only 2nd PH, however, decreased significantly from 437 ± 73 pmol × min^{-1} at baseline to 334 ± 54 pmol × min^{-1} after 5 hours of saline infusion ($p = 0.005$). The curves for insulin secretion during PT 1 and PT 2 were essentially superimposable (Fig. **3**). The different calculated phases of insulin secretion did not change during the lipid infusion and did not differ between the two FFA profiles (Fig. **4**). The only effect observed was a decreased second phase during the 5-hour saline infusion (78 ± 3% of baseline) vs. a maintained 2nd PH during PT 1 (103 ± 11% of basal, $p = 0.05$) and PT 2 (89 ± 4% of basal, $p = 0.04$) after 5 hours.

Discussion

The novelty of the present approach was, in contrast to previous studies examining the effect of endogenous variations of FFA profiles, to experimentally produce different serum patterns of elevated FFAs. Our approach using 2 different lipid emulsions plus acipimox was evidently successful in generating 2 different FFA patterns. The greatest difference between PT 1 and PT 2 was found in the proportion of polyunsaturated FFAs. PT 1 contained relatively more linoleic and linolenic acid, while PT 2 contained relatively more myristic, palmitic, pal-

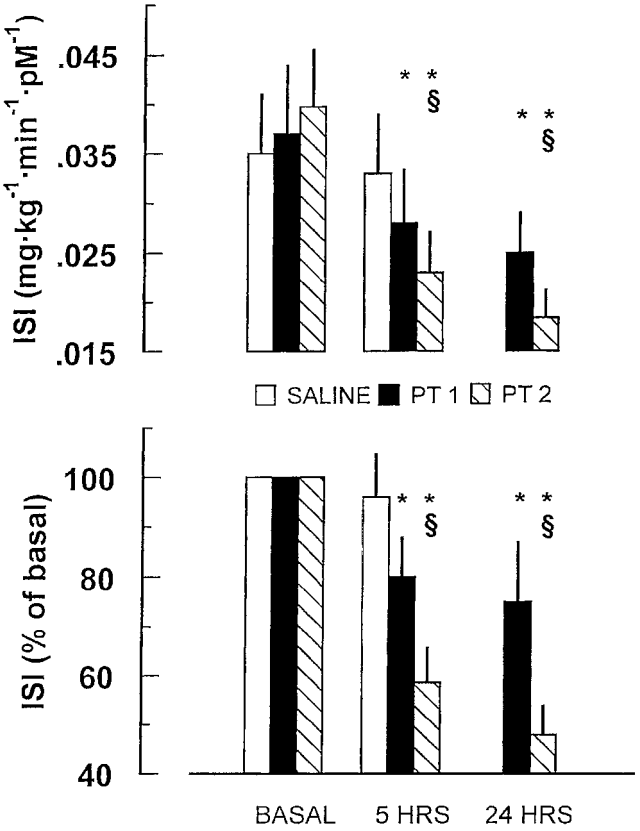

Fig. 2 Insulin sensitivity index (ISI, glucose infusion rate divided by serum insulin) during the lipid and saline infusions (top panel, absolute values; bottom panel, relative change from baseline). * p vs. SALINE < 0.01, § p vs. PT1 < 0.05; p = 0.077 for PT 1 vs. PT 2 (ANOVA).

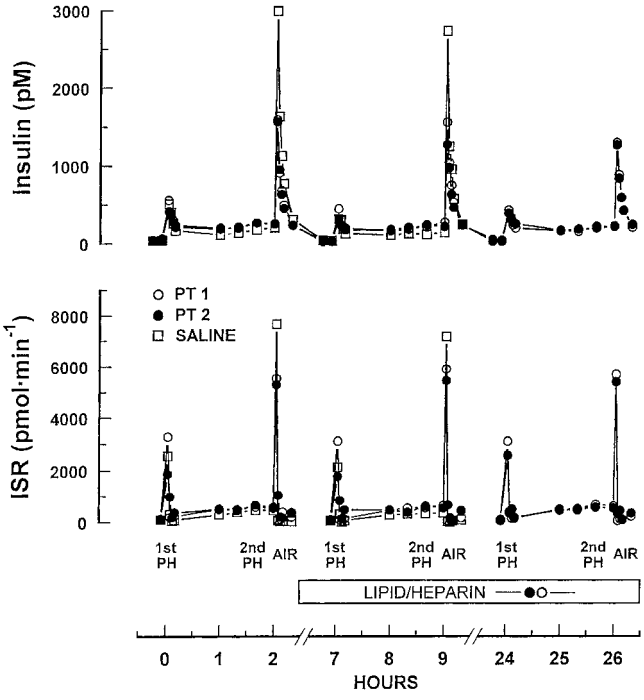

Fig. 3 Insulin concentrations (top panel) and insulin secretion rates (ISR) during the 3 consecutive hyperglycemic clamps performed during the lipid and saline infusions (sem omitted for clarity). 1st PH, first phase of glucose-stimulated insulin secretion; 2nd PH, second phase of glucose-stimulated insulin secretion; AIR, acute response to arginine.

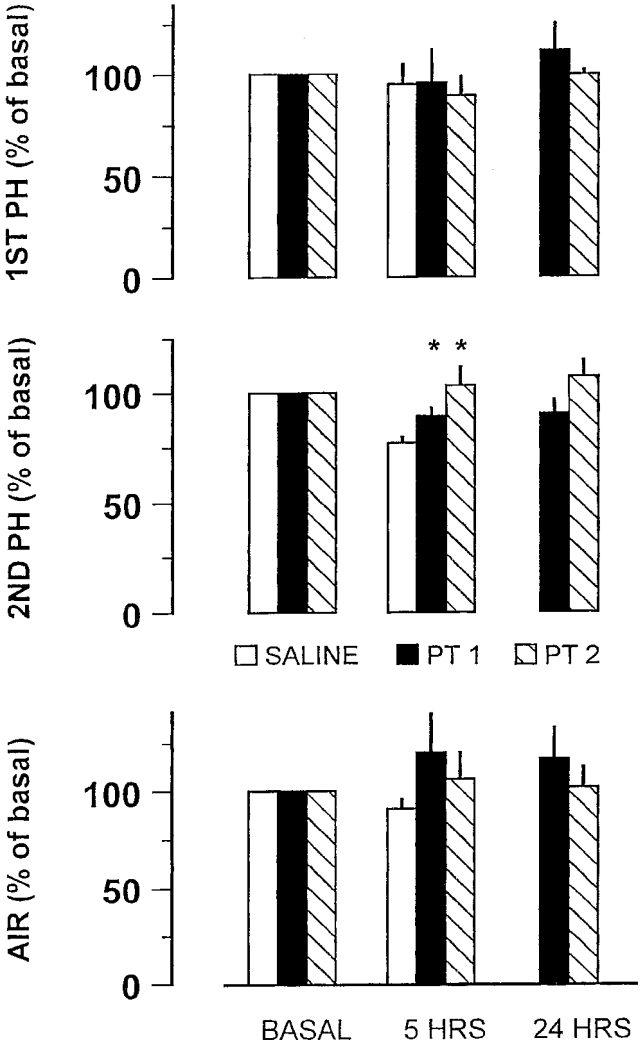

Fig. 4 Different phases of insulin secretion expressed as relative change from baseline: first phase hyperglycemia (1st PH), second phase hyperglycemia (2nd PH), acute insulin response to arginine (AIR). The bars code for the saline protocol (open bars), lipid infusion 1 resulting in pattern 1 (PT1) (solid bars), lipid infusion 2 resulting in pattern 2 (PT2) (hatched bars).

mitoleic and oleic acid. This resulted in a ratio of polyunsaturated to saturated plus monoenes in PT 1 twice as high as in PT 2. Since total FFAs were not significantly different between the 2 protocols, differences in the metabolic effects between the two protocols should solely be the consequence of the different FFA profiles.

Elevation of circulating FFAs resulted in a decrease in insulin-stimulated glucose disposal, that is, development of insulin resistance, confirming numerous previous reports [4]. PT 1, which contained a higher proportion of polyunsaturated FFAs, only resulted in a ~ 25 % decrease of insulin-stimulated glucose disposal in contrast PT 2, which contained a greater proportion of saturated and monounsaturated FFAs, resulting in a ~ 50 % decrease. This finding is consistent with the positive correlation between polyunsaturated FAs and insulin sensitivity found in skeletal muscle [10], the principal organ of insulin-stimulated glucose disposal. Our findings are also in line with

previous studies in animals showing that diets rich in polyunsaturated FAs improve insulin sensitivity [20,21]. It is, however, necessary to mention that the hyperglycemic clamp at whatever glucose level is not the gold standard procedure to assess insulin sensitivity. The reason for our not choosing the euglycemic clamp technique was the possibility of obtaining reasonable estimates for both insulin secretion and insulin sensitivity in one experimental session using the hyperglycemic clamp. Regarding insulin sensitivity, therefore, our results can only be interpreted as preliminary evidence.

It is worth noting that eicosapentaenoic and docosahexaenoic acid in PT 2, both abundant in fish oil, had increased measurably. Fish oil is characterized by a high degree of unsaturation (mainly ω3) and has been shown to improve insulin resistance in animals [20–22] though not in humans [11]. Since PT 2 was associated with poorer insulin sensitivity, our results suggest that the overall proportion of unsaturated FA beneficially influences insulin sensitivity more than the degree of saturation. However, since the proportions of several FAs differed between the two patterns, it is not possible to attribute a metabolic effect to any particular FA.

In contrast to the relatively pronounced effect on insulin sensitivity, no difference was observed for either glucose- or arginine-induced insulin secretion between the 2 FFA patterns. Insulin secretion was also not different during the lipid infusion compared to baseline. The only effect we observed was a greater 2nd PH insulin secretion after 5 hours lipid infusion compared to saline. Since the variation in the insulin secretion rates was much greater than that in insulin sensitivity, a type 2 error due to the small number of subjects cannot be excluded.

While experimental elevation of FFA for 4 or more hours usually produced a marked effect on glucose disposal, results regarding the effect on ISR are by no means consistent. Depending on experimental design, the method to determine insulin secretion and the population studied quite different results have been obtained [6,23–25].

In conclusion, while the proportion of polyunsaturated FAs had a marked effect on insulin sensitivity the same difference in FFA patterns had no impact on insulin secretion. Our findings provide the first evidence, although preliminary due to the small number of subjects and use of the hyperglycemic clamp instead of the euglycemic clamp, that insulin sensitivity is influenced not only by the level of FFAs which was supraphysiological in the present study but also by their composition: the higher the degree of unsaturation, the better for insulin sensitivity. This may have important practical implications for dietary recommendations regarding the proportion of polyunsaturated FAs. While *in vitro* data indicate that an increasing degree of saturation could have offsetting effects on insulin sensitivity and secretion with respect to the overall metabolic benefit, our results suggest that the favorable effect of circulating polyunsaturated FAs on insulin sensitivity is not outweighed by any inhibitory effect on insulin secretion. Potential cellular mechanisms underlying this effect include changes in the membrane lipid composition, interference with intracellular signaling systems and metabolism of the individual fatty acids.

Acknowledgments

We thank all the research volunteers for their participation. We are indebted to Anna Teigeler, Ulrike Schmidt, Sabine Obermüller, Sabine Wolff and Roman Werner for their excellent technical help. We would also like to thank Annette Wochner, a medical student, who helped with many experiments. This study was supported in part by a grant from the European Community (QLRT 1999–00674) to Drs. Stumvoll and grant from the Deutsche Forschungsgemeinschaft DFG (Stu 192/2–1) to Dr. Stumvoll.

References

[1] Bonadonna RC, Bonora E. Glucose and free fatty acid metabolism in human obesity. Diabetes Rev 1997; 5: 21–51
[2] Randle PJ, Priestman DA, Mistry SC, Halsall A. Glucose fatty acid interactions and the regulation of glucose disposal. J Cell Biol 1994; 55S: 1–11
[3] Collier GR, Traianedes K, Macaulay SL, O'Dea K. Effect of fatty acid oxidation inhibition on glucose metabolism in diabetic rats. Horm Metab Res 1993; 25: 9–12
[4] Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997; 46: 3–10
[5] Chen X, Iqbal N, Boden G. The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. J Clin Invest 1999; 103: 365–372
[6] Boden G, Chen X, Rosner J, Barton M. Effects of a 48-h fat infusion on insulin secretion and glucose utilization. Diabetes 1995; 44: 1239–1242
[7] Dobbins RL, Chester MW, Daniels MB, McGarry JD, Stein DT. Circulating fatty acids are essential for efficient glucose-stimulated insulin secretion after prolonged fasting in humans. Diabetes 1998; 47: 1613–1618
[8] Mokuda O, Sakamoto Y, Hu HY, Kawagoe R, Shimizu N. Effects of long chain free fatty acids on glucose-induced insulin secretion in the perfused rat pancreas. Horm Metab Res 1993; 25: 596–597
[9] Pelikanova T, Kohout M, Valek J, Base J, Kazdova L. Insulin secretion and insulin action related to the serum phospholipid fatty acid pattern in healthy men. Metabolism 1989; 38: 188–192
[10] Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med 1993; 328: 238–244
[11] Rivellese AA, Maffettone A, Iovine C, Di Marino L, Annuzzi G, Mancini M, Riccardi G. Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. Diabetes Care 1996; 19: 1207–1213
[12] Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD, McGarry JD. The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. J Clin Invest 1997; 100: 398–403
[13] Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Järvinen H, Evron W, Dailey G, Gerich J. Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. JAMA 1995; 273: 1855–1861
[14] Mitrakou A, Vuorinen-Markkola H, Raptis G, Toft I, Mokan M, Strumph P, Pimenta W, Veneman T, Jenssen T, Bolli G, Korytkowski M, Yki-Järvinen H, Gerich J. Simultaneous assessment of insulin secretion and insulin sensitivity using a hyperglycemic clamp. J Clin Endocrinol Metab 1992; 75: 379–382
[15] Paolisso G, Howard BV. Role of non-esterified fatty acids in the pathogenesis of type 2 diabetes mellitus. Diabet Med 1998; 15: 360–366

[16] Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D, Jr. Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Invest 1984; 74: 1318 – 1328
[17] Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 1986; 27: 114 – 120
[18] Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992; 41: 368 – 377
[19] Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J. Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. J Clin Endocrinol Metab 1980; 51: 520 – 528
[20] Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. Diabetes 1991; 40: 280 – 289
[21] Jucker BM, Cline GW, Barucci N, Shulman GI. Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle: a 13C nuclear magnetic resonance study. Diabetes 1999; 48: 134 – 140
[22] Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. Science 1987; 237: 885 – 888
[23] Paolisso G, Tagliamonte MR, Rizzo MR, Gualdiero P, Saccomanno F, Gambardella A, Giugliano D, D'Onofrio F, Howard BV. Lowering fatty acids potentiates acute insulin response in first degree relatives of people with type II diabetes. Diabetologia 1998; 41: 1127 – 1132
[24] Boden G, Chen X, Iqbal N. Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. Diabetes 1998; 47: 1609 – 1612
[25] Chalkley SM, Kraegen EW, Furler SM, Campbell LV, Chisholm DJ. NEFA elevation during a hyperglycaemic clamp enhances insulin secretion. Diabet Med 1998; 15: 327 – 333

Requests for reprints should be addressed to:

Dr. Michael Stumvoll
Medizinische Universitätsklinik
Otfried-Müller-Strasse 10
72076 Tübingen
Germany

Phone: + 49 (7071) 29-82711
Fax: + 49 (7071) 29-2784